***Module 8. Combining multidimensionality with random regression***

***Step 1. Adding multiple slopes that vary among individuals***

**Sub-goal**: Can we statistically test for individual variation in interaction terms and what sampling regimes might improve the ability to do so?

**Introduction**: Multidimensional phenotypic plasticity is a widespread feature of most living organisms. In many cases it appears to be adaptive. For example, copulating male dung flies stay in copula for a length of time that depends on the size of the female and the abundance of females in the local population (refs), and this fits reasonably well with what we would predict given the fitness gained from staying in copula longer or leaving to search for a new female. For this to have evolved via selection, at some point there must have been among-individual variation reflecting genetic variation for multidimensionality. In other words, one individual probably had a different plane than another. In the module labelled “Multi-dimensional phenotypic plasticity”, you visualized individuals who had reaction norm planes that differed by intercept. Might they differ in slope as well? And, in which dimensions? If you have done the module on random regression (we do highly recommend doing the tutorial on random regression first), you will have some idea of what individual variation in slope means, but extending it to 2 or more dimensions adds some complexity. Here we take you through the process of adding individual variation to different bits of the equation describing a reaction norm plane.

**Exercise**: Let’s start with the equation describing MDPP that ended the module called “Multi-dimensional phenotypic plasticity”.

This equation describes a population average plane in the space described by x1 and x2 with individuals appearing like a stack of paper—individuals differ only in the intercept, which is defined as the point where both x1 and x2 are 0. Whether the plane is warped () or not, all individuals have the same planar shape. If they varied in some aspect of this plane, we would not detect that in this equation.

Let’s add the lessons you learned about random regression in that module to this equation. There, you learned that individuals might also vary in their slope with respect to x1, and that individual slope could covary with individual intercept both positively and negatively. What happens if that exists in the equation above?

The equation becomes:

where

If you did the random regression module, you have seen this before although here we’ve added one subscript to identify that the slopes are with respect to x1. This statement means that the intercept value of the *i* th individual () and the slope of that individual () with respect to x1 are distributed as multivariate normal with means for each attribute of 0 and a variance-covariance structure of . We then specify the variance-covariance structure in a 2 by 2 matrix. is the measure of how much and covary together.

We will simulate data with these terms and assess one consequence of random slopes in a reaction norm plane. First, we will ask where variation due to the random slope exists in the 2 environmental dimensions.

We will start with a population of 100 individuals each measured 20 times in which both x variables were also measured. Both environments are random and unshared.

Below, specify some parameter values:

Individual variance (V’I) =

Measurement variance (VME) =

β1 =

β2 =

β12 =

Individual variance in slope to =

Cov(I,S1) =

It may be useful at first to make the COV(I,S1) = 0, but later you can explore other values.

Once the data are simulated, we can analyze them with R package lmer4 as was done in the random regression module and we will compare the full model to one without or COV(I,S1).

**R code:**

# install.packages("lme4")  
LMM1 <- lme4::lmer(Phenotype ~ 1 + X1\*X2 (1 + X1|Individual), data = sampled\_data)

LMM2 <- lme4::lmer(Phenotype ~ 1 + X1\*X2 (1|Individual), data = sampled\_data)

Statistical output:

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | True | Estimated : Incomplete | Estimated : Full |
| Fixed effects | Fixed effects |  |  |
| Mean of the trait (β0) | 0 | β’0 = ….. | β’0 = ….. |
| Population slope of () | [Put user-entered value of ] | = …. | = …. |
| Population slope of () | [Put user-entered value of ] | |  | | --- | | …. | | |  | | --- | | …. | |
| Population value of parameter for () | [Put user-entered value of ] | …. | …. |
| Random effects |  |  |  |
| Among individual variance in intercept (VI) | [Put user-entered value of ]= …… | Sampled among-individual variance in intercept (V’I) = ….. | Sampled among-individual variance in intercept (V’I) = ….. |
| Among-individual variance in slopes (random slopes) (VS1) | [Put user-entered value of ]= ….. | - | Sampled among-individual variance in slope (V’S1) = ….. |
| Covariance between individual intercept and slope (COV(I,S1)) | [Put user-entered value of ]= ….. | - | Sampled value of ]= ….. |

The above should illustrate again that leaving something out of an analysis model that exists in the data will produce errors in other terms.

To illustrate what variance the random slopes capture, look at the following graphs:

Graph of individual slopes (based on blup slope and intercept values) laid on top of 3-d graph of population average plane (from population parameters). Should be oriented so that slope variation can be seen, and that the lines are drawn in the vertical plane perpendicular to X2 and through X2=0.

A key feature is that while there is a plane that describes the reaction norm in space defined by X1 and X2, the random slopes for X1 are measured in only one value of X2, where it is 0. Below we present another graph with the reaction norm planes of 3 individuals picked from the data.

Graph of three individual planes defined by individual blubs of intercept and S1 but population parameters of and .

As you can see, these planes vary in only one dimension of the environmental space. Since we did not specify any variation in the reaction to X2, or to the interaction between X1 and X2, everyone is assumed to have the same plane in those directions.

If you try this with several different values for the parameters , and , you will see that these affect the orientation of the plane only in one dimension.

**Exercise**: Now we will add among-individual variation in response to X2. In the phenotypic equation, we simply add to the part that depends on X2.

However, we now have a more complex variance-covariance expression, which is the expansion of all possible combinations of three terms instead of two as before:

Again, let’s simulate some data and visualize the results.

We will start with a population of 100 individuals each measured 20 times in which both x variables were also measured. Both environments are random and unshared.

Below, specify some parameter values:

Individual variance (V’I) =

Measurement variance (VME) =

β1 =

β2 =

β12 =

Individual variance in slope to =

Individual variance in slope to =

Cov(I,S1) =

Cov(I,S2) =

Cov(S1,S2) =

We might recommend you set two of these covariances to 0 to start and explore what the other looks like before giving them all values. Strong covariances, either positive or negative, will be easier to visualize.

Once the data are simulated, we can analyze them with lmer4 as was done in the random regression module. This time we will just fit the full model and visualize the results in a graph.

**R code:**

# install.packages("lme4")  
LMM1 <- lme4::lmer(Phenotype ~ 1 + X1\*X2 (1 + X1 + X2|Individual), data = sampled\_data)

**Results**

Statistical output:

|  |  |  |
| --- | --- | --- |
| Parameter | True | Estimated : Full |
| Fixed effects | Fixed effects |  |
| Mean of the trait (β0) | 0 | β’0 = ….. |
| Population slope of () | [Put user-entered value of ] | = …. |
| Population slope of () | [Put user-entered value of ] | |  | | --- | | …. | |
| Population value of parameter for () | [Put user-entered value of ] | …. |
| Random effects |  |  |
| Among individual variance in intercept (VI) | [Put user-entered value of ]= …… | Sampled among-individual variance in intercept (V’I) = ….. |
| Among-individual variance in slopes (random slopes) to X1 (VS1) | [Put user-entered value of ]= ….. | Sampled among-individual variance in slope (V’S1) = ….. |
| Among-individual variance in slopes (random slopes) to X2 (VS2) | [Put user-entered value of ]= ….. | Sampled among-individual variance in slope (V’S2) = ….. |
| Measurement error | [Put user-entered value of ….. | Estimated residual variance (VR) = …. |
| Covariance between individual intercept and slope (COV(I,S1)) | [Put user-entered value of ]= ….. | Sampled value of ]= ….. |
| Covariance between individual intercept and slope (COV(I,S2)) | [Put user-entered value of ]= ….. | Sampled value of ]= ….. |
| Covariance between individual slope to X1 and to X2 (COV(S1, S2)) | [Put user-entered value of ]= ….. | Sampled value of ]= ….. |

To illustrate now what variance both random slopes produce, look at the following graphs:

Graph of three individual planes defined by individual blubs of intercept, S1, and S2 but population parameters of . If possible, plot the boundaries of the population mean plane for reference.

If you try this with several different values for the parameters , , and the 3 covariances, you will see that these affect the orientation of the plane around the 0 point for both environments, and its height at that spot, but any curving of the plane due to the parameter is the same for everyone.

As you can see, these planes now vary in both dimensions of the environmental space. If you think of the intercept as a pole in this space, the individual slopes cause the planes to wobble or tip in all directions at the top of the pole relative to the population mean plane and the variation in intercept causes the plane to rise or sink at the intercept relative to the population mean plane. What do values of the covariances do? You can think of them constraining the range of plane tipping in just certain directions, and the different covariances cause different constraints.

**Step 2: Adding the random interaction term**

Step 1 above layered on the random slopes for each individual X variable, and these served to tip the population mean plane in various directions in environmental space, much like a circus performer spinning plates at various heights and with variable skill. However, the population mean plane could be warped due to an interaction between the two X variables. Because it appears that such warping is adaptive in real organisms, this would imply that individual variation in the extent of warping is possible. Here we add that last random effect term to the phenotypic equation.

This now expands the phenotypic equation to include three slope terms,, , and , which are the individual deviation from the population slope with respect to , , and the interaction between the 2 x variables, respectively. The full equation is thus:

As above, the addition of another random effect expands the variance-covariance once again. It now is an ugly beast, but if you move through it systematically, you will see it is just a ledger sheet that accounts for every possibility:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
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We will simulate data with these terms, and assess two consequences of random slopes in two dimensions. First, we will ask where variation due to the interaction term ends up in a model that lacks that term. Second, we will try to visualize where variation caused by variation in warping is more likely to be seen.

As the phenotypic equation is getting more complex, we will increase the number of sampled individuals to 500. As before, each individual is measured 20 times for both x variables and both environments are random and unshared.

Below, specify some parameter values:

Individual variance (V’I) =

Measurement variance (VME) =

β1 =

β2 =

β12 =

Individual variance in slope to =

Individual variance in slope to =

Individual variance in slope to [=

COV(I,S1)=

COV(I,S2)=

COV(I,S12)=

COV(S1,S2)=

COV(S1,S12)=

COV(S2,S12)=

As before, it might be easiest to start with 0 covariances (represented as correlations) and add them in individually so you can more easily see what each does.

Once the data are simulated, we can analyze them with lme4 as was done in the random regression module. For example, we can use the following equation in which the individual specific interaction term is omitted:

**R code:**

# install.packages("lme4")  
LMM1 <- lme4::lmer(Phenotype ~ 1 + X1\*X2 (1 + X1 + X2|Individual) + (0 + X2|Individual), data = sampled\_data)

And we will compare those results with the full model:

**R code:**

LMM2 <- lme4::lmer(Phenotype ~ 1 + X1\*X2 + (1+X1\*X2|Individual), data = sampled\_data)

**Results**:

Statistical output:

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | True | Estimated : Incomplete | Estimated : Full |
| Fixed effects | Fixed effects |  |  |
| Mean of the trait (β0) | 0 | β’0 = ….. | β’0 = ….. |
| Population slope of () | [Put user-entered value of ] | = …. | = …. |
| Population slope of () | [Put user-entered value of ] | |  | | --- | | …. | | |  | | --- | | …. | |
| Population value of parameter for () | [Put user-entered value of ] | …. | …. |
| Random effects |  |  |  |
| Among individual variance in intercept (VI) | [Put user-entered value of ]= …… | Sampled among-individual variance in intercept (V’I) = ….. | Sampled among-individual variance in intercept (V’I) = ….. |
| Among-individual variance in slopes (random slopes) (VS1) | [Put user-entered value of ]= ….. | Sampled among-individual variance in slope (V’S1) = ….. | Sampled among-individual variance in slope (V’S1) = ….. |
| Among-individual variance in slopes (random slopes) (VS2) | [Put user-entered value of ] | Sampled among-individual variance in slope (V’S2) = ….. | Sampled among-individual variance in slope (V’S2) = ….. |
| Among-individual variance in slopes (random slopes) (VS12) | [Put user-entered value of ] | - | Sampled among-individual variance in slope (V’S12) = ….. |
| Covariance between individual intercept and slope (COV(I,S1)) | [Put user-entered value of ]= ….. | Sampled value of ]= ….. | Sampled value of ]= ….. |
| Covariance between individual intercept and slope (COV(I,S2)) | [Put user-entered value of ]= ….. | Sampled value of ]= ….. | Sampled value of ]= ….. |
| Covariance between individual intercept and interaction term (COV(I,S12) | [Put user-entered value of ]= ….. | - | Sampled value of ]= ….. |
| Covariance between individual slope to X1 and to X2 (COV(S1, S2)) | [Put user-entered value of ]= ….. | Sampled value of ]= ….. | Sampled value of ]= ….. |
| Covariance between individual slope to X1 and interaction term (COV(S1, S12)) | [Put user-entered value of ]= ….. | - | Sampled value of ]= ….. |
| Covariance between individual slope to X2 and interaction term (COV(S2, S12)) | [Put user-entered value of ]= ….. | - | Sampled value of ]= ….. |
| Residual variance (VR) | [Put user-entered value of ] | Sampled residual variance (V’R) = ….. | Sampled residual variance (V’R) = ….. |

As usual, omission of a key parameter causes variation to be placed elsewhere in the equation, in this case mostly in the residual.

A 3-d plot of the population mean (below) provides you with a visual orientation to the average phenotype across the environmental space created by the two X variables. We have also plotted the values different individuals will express at the corners of the graph where they would experience an extreme in both x distributions.

Graphical visualization of population mean plane. [3-d graph with two Xs on horizontal axes and phenotype on vertical axis]. Plot values of each individual at each the corner of the 3d graph in a different color.

Run this simulation several times with different values for the interaction term and the covariance terms. Where does the interaction term create the most phenotypic variance and how do covariances affect this?

**Conclusion**

The effect of interactions between environments on phenotypes has three important characteristics. First, it seems biologically likely given the complexity of the environment and it in fact exists in many traits. Second, these effects can be modelled using mixed models, including the random effects of individual on the response to each environment and in theory on the interaction term itself. The third characteristic is that these models are exceedingly complex. At this point, you don’t have in your mental pocket the full phenotypic equation. There are many more complexities to explore, but you now have all the basic tools. The SQuID platform can now be explored so you can assess what sampling regimes and experimental designs will allow you to effectively measure the attributes of most interest.