***Module 5: Multi-dimensional Phenotypic Plasticity***

In the “*non-stochastic environments”* module, we accounted for the effect of a single environmental factor on phenotype. This statistical process ends up describing the organism’s phenotype as a line in uni-dimensional environment (the environment on the X-axis, phenotype on the Y-axis). For example, parent birds typically increase the rate of food delivery to the nest as their offspring grow older. We call this line a reaction norm, and in the case of parent birds, it makes adaptive sense because older offspring are bigger and need a faster food intake to maintain growth.

A simple reaction norm line in a uni-dimensional environment is a simplified scenario, because we know organisms exist in environments that have multiple environmental factors (e.g., they are multidimensional). There is increasing evidence that multiple factors can influence many phenotypes. In the case of parent birds increasing food delivery to older offspring, maintaining the rate of increase with age over all environmental conditions makes little sense. If, for example, brood size varies among nesting attempts, we might expect parents to respond to both nestling age and brood size. For parent birds, the environment now has two dimensions, nestling age and brood size, and instead of a reaction norm line, their behavioural response could be described by a *plane* in this 2 dimensional space. This is what we have called “multi-dimensional phenotypic plasticity” or MDPP, and in theory an organism could be responding to many environmental factors, so they would have a reaction norm plane existing in n-dimensional environmental space. It is hard for us to visualize past three dimensions, so here we will focus on the behaviour of a reaction norm plane in just two environmental axes, but if you get comfortable with this, it will be easier to imagine three or more environmental axes. Our general goal is to help you gain some understanding of how specific parameter values in an analysis equation influence shape and orientation of a reaction norm plane.

***Step 1. Population level MDPP***

**Sub-goal**: To understand average (population-level) effects of multiple environmental factors on the phenotype.

**Introduction**: Here we model multiple sources of environmental variance (VE) in a single trait, expressed multiple times within individuals but measured within a single population. This step illustrates some differences between simple and multiple regression, but may also allow simulation of more complex data structures, such as correlations between environments (see step 4).

**Exercise**: to explore multiple sources of VE.

Number of individuals:

[Enter]

Among-individual variance (Vi):

[Enter]

Measurement error variance (Vm):

[Enter]

Number of trait expressions measured:

[Enter]

Note: For now, we will assume all individuals are sampled equally often and at the same time.

The environment

Let’s simulate phenotypes that are influenced by two factors, both of which are shared by the whole population (e.g., spring temperature) and the values are random from one measurement period to the next. The environment thus has an intercept effect on phenotype of 0, and a slope that you can input (we recommend at first that the slope be >> 0). Each environment contributes the value [slope]2Var(X) to the total phenotypic variance (see Table 1 in Allegue et al. 2016), so by specifying the slope (positive or negative), you will affect the total phenotypic variance. Note that in SQuID each environmental effect is standardized (i.e. mean = 0 and variance = 1).

Enter the slope for each environmental factor. These can be either positive or negative.

Environment 1:

[Enter]

Environment 2:

[Enter]

< Run simulation>

**Results**

Suppose we assume that there is only one environmental effect. That is, we analyse the population we simulated using the following model:

A mixed statistical model estimates the parameters:

Statistical output:

|  |  |
| --- | --- |
| Estimated | True |
|  |  |
| Individual variance (V’I) = ….. | Individual Variance (VI) = …… |
| Residual variance (V’R) = ….. | Measurement variance (Vm) = ….. |

This makes the simple point, also made in Module “*non-stochastic environments*”, that leaving out an important factor inflates other variance components. In this case it was mostly the residual variance because the environment was set as random from one measurement to the next and all individuals experienced it.

A reanalysis with the following model pulls the missing environmental variance out of the residual term:

Statistical output:

|  |  |
| --- | --- |
| Estimated Value | True Value |
|  |  |
|  |  |
| Individual variance (V’I) = ….. | Individual Variance (VI) = …… |
| Residual variance (V’R) = ….. | Measurement variance (Vm) = ….. |

This is a multiple regression within a mixed model. A 3-dimensional graph helps visualize the way in which the two x variables affect a phenotype in the 2 dimensions defined by the environment.

Graphical visualization of population mean plane drawn from estimated parameter values using full model. [3-d graph with two Xs on horizontal axes and phenotype on vertical axis].

Individuals in this simulation vary in their intercept by the amount you entered previously in VI. Below we pick three individuals across the range of the intercept variance to illustrate how each individual’s plane sits in the space defined by the two environmental variables. You can see that the three planes are parallel or very close to parallel, and differ only in their elevation. If you play around with the number of measures within an individual, you will see that the resolution of these planes requires fairly large sample sizes (this is covered in more detail later).

New 3-d graph with three individuals picked from the low end of I, the middle, and from the high end of I.

Figure

Run through this simulation several times using different values for and , including having some slopes negative. In particular, try making the two have opposite signs. Inspect the table above and look at the two graphs so you gain a feel for how the two slopes produce a flat plane that may be tilted in various ways.

**Conclusion**

This exercise should reinforce your understanding of where measured and unmeasured sources of variance end up in a statistical analysis and how systematic effects of multiple environments can be appropriately captured. In the next step, we illustrate one important complexity.

***Module 5, Step 2. Interaction terms***

**Sub-goal**: To account for dependencies in the effect of one factor by another factor.

**Introduction**: In step 1 above, we developed the idea that 2 or more environments might both have effects on a phenotype. In the case of parent birds feeding offspring, both offspring age and the number of offspring were hypothesized to have effects on a parent’s feeding rate. The analysis in Step 1 produced a flat plane in 2-dimensional environmental space that then could possibly be tilted in a variety of ways depending on the slopes to both environments. The effect of brood size and nestling age is an intriguing case because the increase in need as a nestling gets older is not merely added to the effect of brood size, but rather brood size multiplies the effect of nestling age (the effect of age for 4 nestlings is 4 times the effect for 1 nestling). More generally, we might expect plasticity to one environmental variable to be plastic in the face of other environmental variables. This plasticity in response to one environment of a reaction norm to another (plasticity of plasticity) is a fascinating potential consequence of multidimensional environments.

So far, you have explored the effect of two environmental factors that produce a flat plane. The second statistical equation in step 1 illustrates these as additive effects of the two environmental factors. Put another way, the effects of the factors were treated as independent. Note the distinction between independence of effects and independence of the factors themselves—weak to modest correlations among the factors themselves do not change the independence of the effects of those factors, as will be illustrated in a later module. However, high correlations between factors (colinearity) can have unusual effects on statistical tests of parameter estimates—we ignore that issue for now. Instead, plasticity of plasticity as described above results from non-additive effects. The factors are said to interact, the term describing them in a mixed model or any related analysis (GLM) is an interaction term. Biologically, interactive effects on a phenotype between 2 environmental factors may have multiple fascinating implications.

**Exercise**:

As in step 1, we need to simulate a population and the data we collect from that population.

Number of individuals:

[Enter]

Among-individual variance (VI):

[Enter]

Measurement error variance (Vm):

[Enter]

Number of trait expressions measured:

[Enter]

Note: For now, we will assume all individuals are sampled equally and at the same time.

The environment

Enter the slope for each environmental factor and the interaction term. These can be either positive or negative.

Environment 1:

[Enter]

Environment 2:

[Enter]

Interaction:

[Enter]

< Run simulation>

**Results**

Let’s analyze this simulated population by omitting the interaction term first. In the first case we assume the following statistical model:

The full statistical model including the interaction is:

Here the results compared:

Statistical output:

|  |  |  |
| --- | --- | --- |
| Estimated Values (ignoring interaction) | Estimated Values (from full model) | True Value |
|  |  |  |
|  |  |  |
| - |  |  |
| Individual variance (V’I) = ….. | Individual variance (V’I) = ….. | Individual Variance (VI) = …… |
| Environmental variance accounted for = ….. | Environmental variance accounted for = ….. | Environmental variance (VE) = …. |
| Residual variance (V’R) = ….. | Residual variance (V’R) = ….. | Measurement variance (Vm) = ….. |

You should find from the above that the variance caused by the interaction term, when that term is omitted, ends up mostly in the residuals, although some may end up elsewhere due to sampling issues.

You can visualize the impact of the interaction term in the graph below. Here we have graphed the population average plane derived from the parameter estimates in the simulated data in the space defined by both environments.

Graphical visualization of population mean plane based on parameter estimates. [3-d graph with two Xs on horizontal axes and phenotype on vertical axis].

Examine this graph carefully. The plane produced should look different than the ones you produced in step 1. Those planes were flat but tilted in various ways. If is not 0, the plane in this graph should look warped or bent. This is the influence of the interaction term.

Now redo the above and manipulate both the magnitude of the interaction between x1 and x2 () and its direction relative to the other slopes to assess how this affects your results if you leave it out of your statistical analysis.

You can also see in the graph how the parameter changes the warping of the plane.

**Conclusions**

Multidimensionality of environmental effects on phenotypic attributes is very likely. It may seem redundant to keep demonstrating that leaving out an important term causes that variance to end up in other terms. The unusual element of interactions is that the direction of the slope compared to the main effects matters also. Moreover, interaction terms generate an array of interesting biological questions about both the way organisms integrate information about environment and the selective forces shaping the reaction norm plane.