**Partitioning variances: Differences between Vp, VI and measurement error variance.**

**Goal:** to develop understanding of hierarchies in variance when individuals express traits repeatedly.

***Step 1. Mean and variance when the trait is expressed once and does not change***

**Sub-goal**: Illustrating the concept of variance and mean, for traits with no within-individual variance but which is measured with error.

**Introduction**: This is the simplest possible situation. A trait as measured for a group of individuals from a population is characterised by a mean (*µ*) and a variance (VP).

**Exercise**: In this situation we assume that we only have one value of the trait for each individual. You first have to decide how many individuals to measure.

Number of individuals =…….

In every case that we measure something, we know that we are making an error in the measurement. This error is assumed to be non-directional and hopefully represents only a small portion of the total variance VP. Below play with the error term. Generally measurement error variance should not be high, ideally lower than 5% of the total variance, but of course some traits can be associated with much higher measurement error.

An explanation of notation: There are several kinds of unaccounted variance in a statistical model. This are called, variously, “error variance” or “residual variance”. Measurement error is one source of this variance, but as you will see below, not all unaccounted variance is the result of error. To distinguish these with notation, we will use “Vm” for measurement error and VR for the more general residual variance. In this particular step, VR = Vm.

Note that in this module, the total phenotypic variance (VP) is restrained to 1. This will allow a better understanding of the proportions of the different model variance components.

Vm =…….

GRAPH

|  |  |
| --- | --- |
| True | Estimated |
| Total phenotypic variance (VP) = 1 | Total sampled phenotypic variance (V’P) = ….. |
| Individual variance (VI) = …… |  |
| Measurement error variance (Vm) = ….. |  |
| Mean of the trait () = 0 | Sampled mean of the trait (’) = ….. |

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**Background calculations:** (this part is only used by us to see how each element is calculated. Users don’t see this)

Equation:

Results:

|  |  |
| --- | --- |
| True | Estimated |
| Total phenotypic variance (VP) = 1 | Total sampled phenotypic variance V’P = |
| Individual variance (VI) = |  |
| Residual variance (VR) = Input |  |
| Mean of the trait () = 0 | Sampled mean of the trait (’) = |

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**Point**: The variance of measures (V’P) is often higher than the variance of true individual variance (VI) if there is measurement error (and there will always be some). The estimate of total variance includes both individual differences (VI) (not measured directly) and measurement error (Vm). Note also that the estimated V’P (total variance of sampled values) typically differs slightly from 1. This is because we have sampled from a population where the true total phenotypic variance that includes individual (biological) variance and measurement error variance, while equal to 1, may not be 1 in your sample. The difference thus arises from sampling variance.

**Solutions**: First work to reduce the measurement error; to do so, you need to know the magnitude of measurement error. This requires measuring individuals more than once. This is explained in step 2 of this module +++++

**Statistical model**

Throughout these modules we will provide you with the statistical model that we’ve explored. These come in two forms. The first is an equation that describes each data point (particular measurements on individual phenotypes). Since we are using phenotypic measures as the focus here, we call this the “phenotypic equation”. You have just explored the following phenotypic equation:

where is the measured phenotypic value of the hth measurement (in this case h = 1) on the ith individual, is the true deviation of the individual’s trait from the population mean (which is assumed to be 0 for now),and is the residual deviation of that measurement from the true value, caused in this scenario by measurement error.

The second type of equation is a partitioning of variance where the terms are defined as above.These have direct connection to the values in the phenotypic equation, with the exception that in this scenario the variance in, often called residual variance, is measurement variance **(**

***Step 2 Repeatability and measurement error***

**Sub-goal**: Learning how to estimate measurement error variance (**)** in traits varying solely among individuals.

**Introduction**: In the previous step, you estimated the mean and the total variance of a sample of individuals measured once. From this you learned that some of the observed variation in measures might be due to measurement error, which occurs when the measured value deviates (for whatever reason) from the biological value. Here we detail the sampling design that you might use to directly estimate the magnitude of measurement error; we do this for a trait where the true value of the trait is constant for an individual (cf. a ‘fixed’ rather than ‘labile’ trait, e.g. structural size in adulthood). Measurement error can be estimated by measuring the same set of individuals multiple times (preferably in a blind and randomized order). Since their trait values are fixed, any deviation in measured values across measurements of the same individuals should be due to measurement error.

**Exercise**: To simulate this new situation, we advise using the same parameters as in the previous step, in terms of the number of individuals and measurement error (; expressed as a proportion of the total phenotypic variance, VP) so that you can compare your output with the previous step:

Number of individuals : ……

: ……..

You also need to enter how often each individual is measured. We call this variable “Number of trait expressions”. For simplicity, we are assuming that each individual is measured the same number of times. Because we are interested in setting up a scenario where individuals are assayed repeatedly, you should set a number equal or greater than two. Play around with how often you measure each individual, because the number of repeated measurements per individual affects how well your estimated values approximate the true values.

Number of trait expressions: ……..

Invisible: Number of time steps (100)

Press “RUN” here.

Output

As before, we present the output in the form of a collection of figures and tables that shows you your estimated values (derived from a univariate mixed-effects model) as well as the true values. Compare how the estimated values deviate from the true values; play for example with the ‘number of trait expressions’ to see how the number of repeated measurements per individual affects your estimates and how much they deviate from the true values.

Three graphs here:

1. A histogram that plots the observed values (V’P)
2. A histogram that plots the distribution of individual means (V’I)
3. A histogram that plots the deviations of each observation from the relevant individual’s mean (V’m)

The three density plots show the distribution of estimated total phenotypic variance (V’P), the estimated variance among individuals (V’I), and the estimated variance within individuals (V’m). It demonstrates graphically that V’P = V’I + V’m.

A mixed statistical model estimates these parameters:

|  |  |
| --- | --- |
| True | Estimated |
| Total phenotypic variance (VP) = 1 | Total sampled phenotypic variance (V’P) = ….. |
| Individual variance (VI) = …… | Sampled individual variance (V’I) = ….. |
| Measurement error variance (Vm) = ….. | Measurement error in sample (V’m) = ….. |
| Mean of the trait () = 0 | Sampled mean of the trait (’) = ….. |

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**Background calculations:** (this part is only used by us to see how each element is calculated. Users don’t see this)

Equation:

Mixed effect model (R code):

LMR <- lmer(Phenotype ~ 1 + (1|Individual))

RANDEF <- as.data.frame(VarCorr(LMR))$vcov

Results:

|  |  |
| --- | --- |
| True | Estimated |
| Phenotypic variance (VP) = 1 |  |
| Individual Variance (VI) = 1 - Vm |  |
| Residual variance (VR) = input |  |
| mean of the trait () = 0 |  |

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**Point**

If you have inputted the same parameter values as for step 1, you see that we are now (more) properly estimating the individual variance. This is because we have directly estimated the within-individual variance due to measurement error by measuring each individual (at least) twice.

Measurement error can obscure true biological variance. We typically express the amount of biological variance we can measure as a proportion of the total observed variance; this standardized metric is called “repeatability”:

R = V’I / (V’I + V’m)

Your repeatability is R = …..

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**Background calculations:** (this part is only used by us to see how each element is calculated. Users don’t see this)

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Repeatability tells you how well your current measurement predicts a future measurement of the same individual. Logically, the repeatability is 1 if there is no measurement error (try it out!). The effect of measurement error can be nicely illustrated in a graph where we plot the first vs. second measurement.

Scatter plot

The scatter plot shows how correlated the first and second measurements are. In the absence of measurement error (i.e., Vm = 0), all points align on a single line (y=x). Play around with the settings of Vm; the higher the value, the more widely the data points get dispersed around y=x, i.e. the higher the measurement error the lower our ability to predict an individual’s phenotype based on a previous measurement. Repeatability thus provides an assessment of how well one has measured a particular phenotype. Repeatability can be used for several other purposes too, so the way it is calculated may vary some. We will develop some of these nuances later on.

**Statistical model:**

**R code:**

# install.packages("lme4")

LMM<- lme4::lmer(Phenotype ~ 0 + (1|Individual), data = sampled\_data)

***Step 3. Within- and among-individual variance***

**Sub-goal**: to illustrate hierarchical structure of variance when individuals express traits multiple times (a trait varies within-individuals).

**Introduction**: Individuals express different values of a trait at different times due to the influence of the environment. Collecting two or more measures for each individual provides several types of information. We have already seen that it can allow estimation of measurement error. If the measurements are spread over time, then you can also estimate how much variance is due to the environment and how the phenotype responds to the environment. Finally, repeated measurements can measure whether differences among individuals are solely due to differences in the environment at the time of measurement versus to some other source, such as differences among individuals that were generated before the period of measurement.

**Exercise**: As in the two previous steps we will generate a new group of individuals but we will also add a new form of variance. Previously, you generated a population of individuals that varied in their true value (VI) and also due to observer measurement error (Vm). We assumed that individuals had the same trait value throughout the duration of our study. But, what if trait values changed over time, perhaps in response to some environmental gradient? To start exploring this idea, let’s assume that all individuals would respond to this gradient in the same way, but they might experience different values of the environment. This means that in addition to VI and Vm there is variation caused by the population mean response to an environmental effect on the phenotype of an individual. In this step, the environmental effect is considered as individual-specific but unknown to the observer. An (individual-) specific environmental effect means that each individual of the population will experience different environmental conditions than do other individuals. For instance, the intensity of intra- and inter-specific competition within a population might be experienced differently between individuals. An unknown environmental effect represents environmental values that are not measured thus cannot be included in the statistical analysis. Earlier we noted that VR is called “residual variance”. Now, unknown, environmentally-caused variance will be combined with Vm to make VR , and is the primary reason for calling it “residual” variance.In reality, there may be many environmental effects, both known and unknown. We will use the term VE to refer to all phenotypic variance caused by the environment. Known environmental variance will be indicated by Vβx.

In this simulation, let’s assume there is only one environmental effect. We will specify it to generate phenotypes, so let’s use the term Vβx. You can thus try several combinations of Vm, VI, and Vβx (with the constraint that they add up to 1) to uncover how the input values affect the estimates of repeatability and each variance component. Just remember that for now Vβx is one environmental effect but it will be measured as part of the residual.

Number of individuals =…….

Vm =…….

VI =…….

Vβx = ……. [Note that the slider specifies both the variance due to x as well as the slope since SQuID uses a standardized variance of 1 for x].

Number of trait expressions = ……

5 GRAPHS here

A mixed statistical model estimates these parameters:

|  |  |
| --- | --- |
| True | Estimated |
| Total phenotypic variance (VP) = 1 | Total Phenotypic variance in sample (V’P) = ….. |
| Individual Variance (VI) = …… | Sampled Individual variance (V’I) = ….. |
| Residual variance (Vβx + Vm) = ….. | Residual variance of sample (V’R) = ….. |
| mean of the trait () = 0 | Sampled mean of the trait (’) = ….. |

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**Background calculations:** (this part is only used by us to see how each element is calculated. Users don’t see this)

Equation:

Where

since Vx = 1 in Squid.

Mixed effect model (R code):

LMR <- lmer(Phenotype ~ 1 + (1|Individual))

RANDEF <- as.data.frame(VarCorr(LMR))$vcov

Results:

|  |  |
| --- | --- |
| True | Estimated |
| Population Phenotypic variance (VP) = 1 |  |
| Individual Variance (VI) = input |  |
| Residual variance (Vβx + Vm) = Vm (input) + Vβx |  |
| mean of the trait () = 0 |  |

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Once again you can calculate repeatability as

R = V’I / (V’I + V’R)

Your repeatability is R = …..

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**Background calculations:** (this part is only used by us to see how each element is calculated. Users don’t see this)

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**Point**: We can now estimate a particular variance component of VP that represents among-individual differences that are consistent through time. This variance is also, in some circumstances, the index for individual “personality” differences. The residual variance combines both measurement error and the variance caused by the unmeasured (unknown) specific environment to each individual. VE in general reflects plasticity to unknown environments, with Vβx indicating variance due to a specified environmental variable (x), which so far has not been measured.

You can now test whether the number of individuals sampled and the number of repeated measures per individual can affect your estimation.

Repeatability now does not represent the same thing as previously because the denominator of the ratio includes both measurement error and variance due to plasticity. This new repeatability is not simply a measure of your skill at measuring phenotypes. Instead, it now is an estimate of a biological phenomenon: consistent individual differences (i.e. personality in the case of behaviour). This estimate is conservative because measurement error reduces the estimate to be less than the true biological repeatability. Measurement errors in this scenario are not separable from the plastic response of individuals to an unmeasured environment. To calculate measurement error alone, you would have to collect more than one measure on the same trait for each individual in the same environment (e.g. two persons can observe the behaviour of an individual on the same video recording or measure the trait at close to the same time).

**Statistical model**

Because the environmental effect in this scenario was unknown, the statistical models are the same as before, with two exceptions. First, the phenotypic equation is as before,

except that contains both measurement error and deviations described by

The variance equation is now

where

**R code:**

# install.packages("lme4")

LMM <- lme4::lmer(Phenotype ~ 0 + (1|Individual), data = sampled\_data)

***Step 4. Explaining Environmental Variance***

**Sub-goal**: to explain unknown environmental variance

**Introduction**: In step 3, we introduced Vβx as the variance caused by the environment. We did not know what that effect was (it was unmeasured), but often we can measure the environment and assess its influence on phenotype. In this step, we demonstrate how that is done.

**Exercise**: As before, we will generate a new group of individuals, with phenotypic variance caused by measurement error (Vm), individual differences (VI), and specific and measured (i.e. known) environmental effects (Vβx) on the phenotype of an individual.

As before, you can set Vm, VI and Vβx, but we suggest you use the same values you did in Step 3. Remember that these variances must add up to 1.

Number of individuals =…….

Vm =…….

VI =…….

Vβx = …….

You can also set the number trait expressions = […..]

At this point, we want to expand on the idea of statistical models. The equations that specify effects producing each individual data point can be hypotheses about the real world. In the made-up world of SQuID, these analysis models have the potential of recreating it exactly. The real world is different and most of the lessons we will cover have to do with problems in estimating terms in analysis models when much is unknown. For now, we will specify a model that should recreate our simulated set of effects completely (with the caveat that we are sampling from an infinite population so observed values will differ from input values). This new model will make explicit as well that is defined as the deviation of each individual from a population value. Until now, that population value has implicitly been 0, so we haven’t used it. But, since we are now including a slope term that allows us to explain environmental variation, it is important to also introduce the population intercept term. For now, we will still have the population mean be = 0, but it is appropriate to include this intercept in all equations because it could be some other value. The model is:

where is the phenotype measured at the *h*th time for the *i*th individual, is the population mean phenotype, Ii is the individual mean deviation from the population mean for the *i*th individual, 𝛽 is the population mean effect of measured environment on the measure of phenotype, and *e*hi is the error made in that measurement.

Note that in SQuID each environmental effect (x) is expressed in unit variance (i.e., Var(x)=1) and mean-centred (E(x)=0). Then .

A mixed statistical model estimates these parameters:

|  |  |
| --- | --- |
| True | Estimated |
| Population intercept () = 0 | Population estimated mean () = … |
| Individual variance (VI) = Input | Individual variance in sample (V’I) = ….. |
| Residual variance (VR) = Input | Residual variance of sample (V’R) = ….. |
| Mean of the trait () = 0 | Sampled mean of the trait (’) = ….. |
| Slope of environmental effect (β) = Input | Estimated slope of environmental effect (β’) = …. |

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**Background calculations:** (this part is only used by us to see how each element is calculated. Users don’t see this)

Equation:

Mixed effect model (R code):

LMR <- lmer(Phenotype ~ X1 + (1|Individual))

FIXEF <- fixef(LMR)

SE.FIXEF <- se.fixef(LMR)

RANDEF <- as.data.frame(VarCorr(LMR))$vcov

Results:

|  |  |
| --- | --- |
| True | Estimated |
| Individual variance (VI) = input |  |
| Residual variance (VR) = input |  |
| mean of the trait () = 0 |  |
|  |  |

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Visually, you can see what β1 is in the following graph:

Insert graph of Phenotype (Y axis) and Environment () on x-axis, with line through scatterplot of points. Output “β’1 = …+/- SE” and “β1 = …” on graph.

The variance among individuals can still be visualized in this world by plotting each individual’s dataset and line: Insert plot of individual reaction norms with VI indicate by a bracket at E = 0.

We encourage you to go back and play around with the magnitude of Vm to see how it affects estimates of β and VI. You can also play around with the slope and the ratio of Vβx and VI to better understand the effects.

**Point**: This exercise introduced explanatory variables (also known as fixed effects). Because individual is a “random” effect, this is thus a “mixed effects” model. The fixed effect part is a linear regression. Even if this is all you want to do with your data, it is important to understand that a sampling regime in which individuals are measured more than once creates the need to do linear regression within a mixed model. Although we do not focus much on significance testing here, the structure of data collected in this simulation strongly affects inferences based on hypothesis testing. More importantly, the combination of random effects and fixed effects sets one up to investigate a wide array of processes involved at one or more levels in this hierarchal structure of among versus within-individual variance.

**Statistical model**

**R code:**

# install.packages("lme4")

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