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Variance between individuals can provide additional information for ecotoxicologists besides the mean

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In dose-response studies, the standard method is to compare the means of the tested parameters of the treated groups to the control. The variance is usually considered as background noise. Our example demonstrates how variance is not just a confounding factor, but can be a source of meaningful information. We measured fifteen parameters to identify which ones show differences in variance related to concentration.

Ecotoxicologists usually think about variance as a nuisance

Ecotoxicologists usually consider variance (the distribution of data around the mean) as a nuisance. It causes several setbacks, like simple statistical tests cannot be used, our estimates become imprecise, and differences from the control group become hard to find. However, in the 1980s and 1990s, discussion emerged around the use of variance as a meaningful parameter (*1,2*) which could even be more sensitive than the “golden” mean (*3*).

An experiment was performed to assess the effect of increasing insecticide concentrations on the springtail (*Folsomia candida*). Our model animal was the “white-rat” springtail, a soil living animal microorganism, which is asexual and very common in organic matter rich soil. Several standard tests (*e.g.* OECD, ISO) were developed using them. Springtails have a role in the litter decomposition and regulation of soil microbes, such as mycorrhiza.

One of the main reasons behind changes in variance may be selection; selection may cause the genetic variance to decrease. On the other hand, with basic high genetic variance, toxic material could induce different reactions in different genotypes. Asexual species show little genetic variability; however, epigenetic variation can provide the raw material for selection in their populations. Epigenetic modifications change the DNA expression without altering DNA itself.

The experiment about variance

A multigeneration experiment was performed with the springtail. The animals were treated with increasing concentrations of an insecticide Trebon 30 EC (*4*). Three growth, eight reproduction, and two behavioural parameters, and the food consumption were measured, and the growth-reproduction trade-off calculated (*5,6*). Measurements were repeated on the three subsequent generations in two ways: (i) the offspring got the same treatment as the parents (multigenerational treatment) and (ii) the offspring were not treated (transgenerational treatment). Only the effects of the parental, grandparental, grand-grandparental treatment were visible in the subsequent generations (see **Figure 1**). Variance differences from the control were then statistically tested.

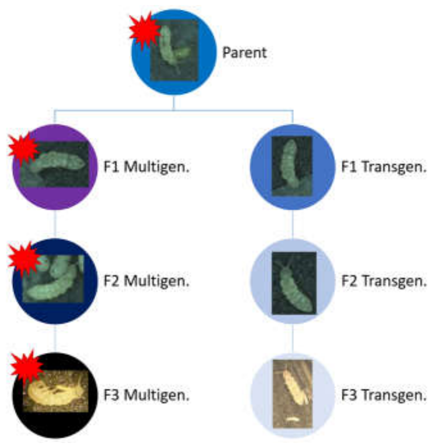

Figure 1. *Experimental design. The darkening colour symbolises the strengthening effect of the insecticide and the fading colour the weakening effect. The red spark shows the insecticide treated groups*

Variance reveals different selection types

In one-third of the parameters, we found a significant change in variance (decrease, or increase), compared to the control. In the following, we present the results for the first clutch size (an important life-history parameter), and explain the possible reasons behind the observed variance changes.

In the first generation, the variance increased in the treated groups compared to the control, which can have several causes. Trait-flexibility could be one reason, but if that were true, then we would not see an effect in the transgenerational group, which was not treated; only maternal effects would be present. More variable offspring have a better chance to survive in a stressful and changing environment, because at least some of them will survive the new circumstances. In addition, selection can support not only one but more strategies simultaneously; this is called disruptive selection. As a theoretical example, there are two strategies found in case of springtails. Those who lay few eggs during the first reproduction (*e.g.* 20) and detoxify more; or those who lay a lots of eggs during their first reproduction (*e.g.* 60) and detoxify less, compared to an intermediate strategy, where the mean is stable at 40 eggs and has a low variance (*e.g.* 10). Thus, for the whole population the mean number of eggs is still 40, but because half of the population tends to a mean of 20 and the other to 60, the variation becomes 30, which is much higher than the original 10.

In our experiment, the two possible successful strategies are (i) individuals invest energy into the first clutch, but not much into detoxification, and (ii) individuals invest energy into detoxification and their own survival, but not into the size of the first clutch. Both strategies may be successful in the long-term for the survival of the population. This energy trade-off was observed with fruit-flies and mosquitos (*7,8*). However, disruptive selection is a low probability explanation, while it acts in the long-term and the next generations show a different pattern, the increasing variance may indicate maternal effects. In addition, it is known that the next generation embryonically may be affected in the body of the adult animal.

In the later generations, the variance of the first clutch size decreased because of the treatment, while the mean of the parameter did not change. This pattern is the marker of stabilising selection when the mean of the given trait is near the current best possibility, and that is why individuals farther from the mean are selected out. Another possibility is that producing variable offspring for the stressful environment is costly for the animals, so it will not be worth keeping up the process if the stress is not too high. The third option is that because one reaction is the best in a particular stress situation, the phenotypic variance, so the variance of the appearance of trait is channelled into a narrow range. In the case of water fleas, it has been found that high concentration treatment with crude oil could decrease the variance compared to the control (*9*).

As such, we conclude that variance can give information about the stress state of animals, even when the measured mean does not react; as such, variance may provide early warning of problems. In addition, testing variance differences does not require extra laboratory work or measurements, only further calculations on existing data.

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