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The inter-individual variance can provide additional information for the ecotoxicologists beside the mean

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ABSTRACT

The hypothesis that the inter-individual parameter variability is an unexploited area of ecotoxicology was proposed several decades ago. Although some illustrative examples were presented to support this hypothesis in the last decades, it has never been tested on an extensive, coherent database. In this study, variance changes of 105 dose-response curves were analysed. All data originated from the same experiment, where the effects of the insecticide Trebon EC were investigated in a dose-response manner on 15 traits of the collembolan *Folsomia candida* in four subsequent generations and two types of insecticide treatments. A consistent relationship between inter-individual variance and insecticide application was found in 2 (first clutch size and growth-reproduction trade-off) out of the 15 of the parameters. Contrary to the mean, the variance of the first clutch size showed consistent differences compared to the control. Furthermore, the variance of the growth-reproduction trade-off was consistently different from the control except in one case (F3 generation of the transgenerational treatment). Higher first clutch size variances were found in F1 and a lower one in the F2 and F3 generations than in that of the control. This overall pattern of the variance changes of the first clutch size and the trade-off seems to be a quick response to the insecticide application. In the short term, we have found that variance increased with insecticide treatment (P and F1 generation), because phenotypic variance generally increases due to environmental stress. Disruptive selection could be another mechanism between the more detoxification less reproduction strategy and the more reproduction less detoxification strategy. However, in the later generations (F2-F3) the variance decreases compared to the control, which could be because on short term selection stronger on the viability parameters and in long-term selection on reproduction becomes stronger. According to our results, analysis of the variance changes of some parameters may give information about the effects of the pesticide even when the mean does not predict any impact. Testing variance changes are important in ecotoxicology because variance change can signalise toxicant impact even when the mean does not change in certain cases.

1. Introduction

Performing laboratory tests with an insecticide according to the dose-response model is a standard procedure in ecotoxicology. The main question in these studies is whether the mean of the parameter values in the treatment groups shows the dose-response relationship or not (Bennett, 1987). Calculation of the ECx value is also based on the mean of the parameters in official guidelines (e.g. OECD, 2006).

In laboratory toxicology experiments, the inter-individual variance is regarded as a complicating factor and not as a source of new information (e.g. OECD or ISO standards). Therefore, the uniformity of the tested populations in the genetic quality assurance programs became a central requirement (Benavides et al., 2020; Beynen et al., 2003). The situation is similar in ecotoxicology. The guidelines prescribe the age, genetic

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line, sex of the animals, breeding and feeding conditions, and standardised abiotic environment to arrange uniform experimental plant or animal strains. According to the guidelines for the *Folsomia candida* (ISO, 1999; OECD, 2009), the tested animals should be 10–12 days-old, healthy, well-fed, and in a good physical condition.

However, the variability of a given parameter measured with the variance may consist of relevant information for ecotoxicology, as Bennett (1987) and Calow (1996) drew attention to it several decades ago. Later, Orlando and Guillette (2001) argued that variance could be an even more sensitive parameter than the mean of a parameter. Despite these suggestions, little effort was made so far in exploiting the potential of the variability evaluation in ecotoxicological research. For example, Holloway et al. (1997) study who showed considerably enhance of the variation of detoxification enzyme activity, when the food of the rice weevil (*Sitophilus oryzae*) was changed to a more toxic yellow split-pea. However, variance change was not tested statistically either.

Devin et al. (2014) elaborated three possible patterns of the dose-dependent variance alteration of a phenotypic parameter under sublethal toxic exposure, which are: (i) constant variance of the parameter across all concentrations due to low phenotypic and genetic variance, (ii) decreasing parameter variance with increasing toxicant concentration that can result from canalisation of the response, i.e. individuals develop more similar phenotypes in the presence of the toxicant than in a clean environment, (iii) increasing parameter variance with the toxicant can occur if individuals show different biological responses. In case (ii), the background of the higher baseline phenotypic variability can be genetic or environmental (other uncontrolled environmental factors or epigenetic differences) variability and accordingly, genetic or environmental canalisation can happen (Flatt, 2005). The reason for the response variability in case (iii) can be a cryptic genetic variation of the population released by the effects of the toxicant as a decanalising environment (Flatt, 2005) or environmental (epigenetic) variability.

The prevailing view in ecotoxicology is that there are four main ways to a toxicant to change the genetic variation of natural populations (Bickham, 2011; van Straalen and Timmermans, 2002). A pollutant can influence the genetic diversity by (1) increasing the mutation rate, (2) selecting for tolerant individuals, (3) increasing drift because of reduction or fragmentation of populations and (4) altering migration.

Environmental contamination as a selection force can influence the mean and variance of the different parameters in various ways (Newman and Clements, 2008). Directional selection is mainly affecting the mean of a parameter, but in some cases, it can affect the variance as well (Thoday, 1972). Stabilising selection does not have a substantial effect on the mean but decreases the variance of the parameter (Pélabon et al., 2010). This kind of selection favours the average of the parameter, which is well-adapted to the local environment (Kingsolver and Diamond, 2011; van Straalen and Timmermans, 2002). Disruptive selection can happen if the intermediate parameter has lower fitness under the contamination pressure. In this case, the mean does not change significantly, but the variance of the parameter increases (Roff, 1997; Thoday, 1972), so selection favours both low and high extremes (van Straalen and Timmermans, 2002). Orlando and Guillette (2001) argue that variance can increase as well as decrease due to pollution exposure. Summing up these arguments, it is legitimate to assume that the variance statistics can be a relevant measure of exposure to toxicants.

Recent pieces of evidence suggest that besides the mean, the variance changes of end-points in laboratory experiments may provide relevant excess information about the effect of the environmental disturbances (Nikinmaa and Anttila, 2019). They found that in 5 out of 103 aquatic toxicological papers, treatment affected the variance. That is why they are advocating for the use of variance as an information source in ecotoxicological studies.

There is one more point to analyse the inter-individual variance. The experimental results contain variance data. Consequently, they are available for further statistical analysis without extra effort to obtain more data from the study. Furthermore, information about variance changes could provide an early warning about stress, while it seems to be more sensitive than mean in some cases without much extra effort or cost.

Why is the study of the inter-individual variance a neglected parameter in ecotoxicological studies? Probably because of the hypothesis that the variance of the treated groups is statistically different compared to the control that was not tested widely on comparable, large data sets. Virtually, the only detailed analysis of the above hypothesis in ecotoxicological studies has been performed by Nikinmaa et al. (2019). They found that the water-soluble fraction of crude oil decreased the variance of the oxygen consumption rate of *Daphnia magna* at the highest concentration (30% water-soluble fraction, 48 h of testing) without any effect on the mean. It was pointed out that the highest applied concentration was over the environmentally relevant ones.

Extensive evaluation of the variance changes in dose-response studies is still lacking. As stated above, *F. candida* has several official guidelines (ISO, 1999; OECD, 2009), which shows precisely that this is an important, standard species in ecotoxicology. Therefore, we chose this species to study the importance of testing variance in ecotoxicology. *F. candida* is regarded as a parthenogenetic species (Chenon et al., 2000; Crouau and Cazes, 2003; Hopkin, 1997), but rare sexual linages are described as well (Kampfraath et al., 2020). In ecotoxicology, the parthenogenetic lineages are extensively used as test animal.

Epigenetic effects and epigenotypes could take over similar function as genotype, generating different phenotypes for selection. Epigenetic inheritance may be the basis of selection in asexual (including parthenogenetic) species (Verhoeven and Preite, 2014). Epigenotypes can change their flexible responding to environmental impacts. This flexibility makes the *F. candida* adaptable to new selection factors (Szabó et al., 2019a).

The objective of this study was to investigate whether there are consistent statistical differences in the parameter variances compared to the control or not. We regarded significant changes in the parameter variance as a consistent difference if we found it in every generation in both treatments in at least one concentration. Moreover, we hypothesised that a consistent relationship between variance changes and insecticide applications would occur, at least in some parameters. Our experiment was performed with sublethal insecticide concentrations, including the environmentally relevant ones.

2. Material and methods

The analyses in this study are based on the raw data of the experiment, presented in Szabó et al. (2020), where a detailed description of the study methods is to be found. Briefly, the experiment was performed as follows.

Folsomia candida Willem 1902 obtained from our stock populations at Szent István University, Department of Zoology and Animal Ecology was used as the model animal. They belonged to the B-clade of collembolans (sensu Tully et al., 2006). The collembolans were kept, and the experiment was performed under the same standard conditions as follows: total darkness in the incubation cabinet, where the temperature was $20 \pm 0.2\,^{\circ}C$, with ~100% humidity. The collembolans were fed with dry baker's yeast once per week, ad libitum. As *F. candida* is a parthenogenetic animal, we started the experiment from one synchronised population according to the OECD guideline (2009). We assumed that the starting population was genetically homogeneous, and every variance change was caused by selection on epigenetic differences of the starting population and/or by epigenetic effects of the treatments.

The widely used insecticide Trebon® 30 EC (MitsuiChemicals Inc. America, 2020), with the active ingredient etofenprox (pyrethroid), was applied in the experiment in six concentrations (0, 0.766, 1.303, 2.215, 3.765 and 6.4 ml Trebon 30 EC/L water). The collembolans were arranged individually into Petri dishes filled with a 0.5 cm layer of plaster of Paris and powdered graphite (8:1) mixture (Fountain and Hopkin,

2001). Dose-response experiments were performed in the parent generation and subsequently, three generations. When the juveniles of the parent generation became 10–12 days old, the F1 generations were divided into two treatment groups. In the multigenerational group, all three generations were handled with the six Trebon 30 EC concentrations in a dose-response manner. On the contrary, in the transgenerational group, juveniles of the six parental groups were grown without insecticide applications. This set-up was continued in F2-F3 generations. The animals were handled identically in the filial generations as the parents, and the measurements were carried out in the same way.

Fifteen life-history parameters were measured. Raw data are to be found in Szabó et al. (2019b) (Supplementary Table S1), except that the data of the first clutch size is involved only in the present study. Growth was specified with initial length, final length (both from the front of the head to the end of the last abdomen segment), and absolute growth of the animals (difference of the initial and final length). The following reproduction parameters were measured: total number of eggs, size of the first clutch (number of eggs in the first clutch), number of clutches, time of maturation (egg-laying time of the first clutch), the ratio of egg diameters, egg volume, unhatched ratio, reproduction investment (total number of eggs multiplied by the mean egg volume). The movement velocity of the animals and the movement energy (the total times of movement activity multiplied by mean velocity during movement activity) represented the behavioural parameters of the collembolans (Szabó et al., 2018). Moreover, food consumption and the growth-reproduction trade-off (absolute growth divided by reproduction investment) were also calculated.

We tested whether the variances of the above-described parameters of insecticide-treated *F. candida* collembolan groups differs from the control in our dose-response experiment. Four generations, the parent and two treatments (multi- and transgenerational) in F1–F3 generations (altogether seven groups), and 15 parameters in all treatments and generations were examined. In such a way, all together, 105 dose-response experiments were available for the statistical analyses. It has to be pointed out that the number of replicates is 4–5 in most ecotoxicological studies. We applied as high as 15 and 12 replicates for the parent and filial generations, respectively. All experiments were performed at the same time, under identical circumstances and methods in the same laboratory.

All of the statistical analyses were conducted using the R Statistical program 3.6.3. (R Core Team, 2020). Dose-response analyses were performed for all parameters in every treatment and generation. So, the statistical comparison of the means was available for 105 dose-response curves in our previous manuscript (Szabó et al., 2020). The effects of Trebon 30 EC on the mean of the size of the first clutch was not included in it (Szabó et al., 2020), so this parameter was analysed with general linear models in this study.

The effect of the concentrations on the variances of the parameters was investigated with the delta method (Millar, 2011). The base models were GLS (generalised least squares) models with the following structure: parameter~concentration+0, with a maximum likelihood method from the nlme package (Pinheiro et al., 2013). The natural logarithm of the estimated variances and their approximate covariance matrix were extracted from the output of the model. Based on the delta method, Dunnett's multiple comparison test relating to the estimated variances in the treatment groups compared to those of the control group was carried out with the aid of the R packages MASS, emmeans, and multcomp (Hothorn et al., 2008; Lenth, 2020; Venables and Ripley, 2002).

We note that the delta method applied to the maximum likelihood estimates of the variances is a more powerful parametric test to check the differences between variances than the mostly applied nonparametric Levene's test.

3. Results

A comparison of the variances could not be performed in five of the 105 dose-response curves (Supplementary Material S1–S7) because there was no variance in the clutch size and in the time of maturity in some F2-F3 generation groups (Supplementary Material, Tables S4, S5, and S7). A statistically significant effect was found in 34 out of 100 tested curves, which is about one-third of all possible events (Table 1). Marginally significant results, when the p-value is between 0.05 and 0.06, are included. No consistent difference was found in the variance of most of the parameters (initial length, the total number of eggs, the number of clutches, time of maturation, egg volume, unhatching ratio, food consumption, velocity, and movement energy) across the generations or between the treatments. On the other hand, the variance of the size of the first clutch and the trade-off in treated groups differed significantly from the control in every generation (except the trade-off at F3 generation) (Table 1). Only the statistically significant differences will be presented hereafter.

3.1. Parent generation

Differences between the control and treated groups were found in some cases. The variance was lower compared to the control in the case of the number of clutches at 2.2 ml L^{-1}, the egg volume at 0.77 ml L^{-1}, the food consumption at 0.77 ml L^{-1}, and the movement energy at 1.3 ml L^{-1} concentration. The variance of the first clutch size was higher by a marginally significant level than the control at one concentration (3.8 ml L^{-1}) (Table 2). The trade-off had lower at 0.77 ml L^{-1}, and higher variance at 1.3 and 3.7 ml L^{-1} concentration than the control (Table 3). The variance of initial length, final length, absolute growth, total number of eggs, time of maturation, ratio of egg diameters, unhatching ratio, reproduction investment, and velocity were not significantly different from the control group (Supplementary Material, Table S1).

3.2. F1 generation

In the F1 multigenerational treatment, the initial length at 0.77 ml L^{-1}, the variance of velocity at 6.4 ml L^{-1}, and the trade-off at 0.77 ml L^{-1} and 1.3 ml L^{-1} concentration had a lower variance than that of the control. The variance of the first clutch size was higher in all concentrations than the control (at the highest concentration, the difference was marginally significant) (Table 2). The variance of the time of maturation at 2.2 ml L^{-1}, and the unhatching ratio at all concentrations

Table 1
The table shows the statistically significant difference in the variance from the control group.

	Generation						
Parameter	P	MF1	TF1	MF2	TF2	MF3	TF3
Initial length	ns	*	ns	*	ns	ns	*
Final length	ns	ns	ns	ns	ns	ns	ns
Absolute growth	ns	ns	ns	ns	ns	ns	*
Total number of eggs	ns	ns	ns	*	*	ns	ns
Number of clutches	*	ns	ns	ns	ns	ns	*
Size of the first clutch	*	*	*	*	*	*	*
Time of maturation	ns	*	*	ns	ns	ns	ns
Ratio of egg diameters	ns	ns	ns	ns	ns	ns	ns
Egg volume	*	ns	ns	ns	*	ns	ns
Unhatching ratio	ns	*	*	ns	ns	ns	ns
Reproduction investment	ns	ns	ns	ns	ns	ns	ns
Food consumption	*	ns	ns	ns	ns	*	ns
Velocity	ns	*	ns	*	*	ns	ns
Movement energy	*	ns	*	ns	ns	ns	ns
Trade-off	*	*	*	*	*	*	ns

Notes: *: significant difference was found in case if the difference was found at least in one concentration; ns: no significant difference in any concentration; P: parent generation, MF1: multigeneration, treated F1 generation, TF1: transgeneration, untreated F1 generation, MF2: multigeneration F2 generation, TF2: transgeneration F2 generation, MF3: multigeneration F3 generation, TF3: transgeneration F3 generation.

Table 2
The difference in the variance of the first clutch size.

Concentration (ml/l)	Generation													
	P		MF1		TF1		MF2		TF2		MF3		TF3	
	z	p	z	p	z	p	z	p	z	p	z	p	z	p
0.766	-0.3	0.998	**-4.0**	**<0.001**	**-6.2**	**<0.001**	**-2.5**	**0.050**	-1.1	0.686	2.1	0.110	2.0	0.156
1.303	-0.2	1.000	**-5.7**	**<0.001**	**-3.7**	**<0.002**	**3.0**	**0.013**	-1.0	0.778	**5.7**	**<0.001**	1.9	0.213
2.215	0.04	1.000	**-5.6**	**<0.001**	**-4.2**	**<0.003**	**3.7**	**0.001**	**3.2**	**0.007**	**2.9**	**0.015**	**6.3**	**<0.001**
3.765	**-2.5**	**0.053**	**-4.6**	**<0.001**	**-3.8**	**<0.004**	**3.7**	**<0.001**	-1.3	0.576	0.9	0.761	1.4	0.485
6.4	-1.2	0.597	**-2.5**	**0.055**	**-6.0**	**<0.005**	**3.0**	**0.014**	-0.4	0.996	–	–	**5.3**	**<0.001**

Notes: Significant differences are marked with bold. Abbreviations of the generations can be seen in Table 1.

Table 3
The difference in the variance of the trade-off.

Concentration (ml/l)	Generation													
	P		MF1		TF1		MF2		TF2		MF3		TF3	
	z	p	z	p	z	p	z	p	z	p	z	p	z	p
0.766	**3.1**	**0.008**	**5.4**	**<0.001**	**3.6**	**0.002**	**5.0**	**<0.001**	-1.4	0.494	**2.8**	**0.019**	-1.6	0.372
1.303	**-3.3**	**0.004**	**4.4**	**<0.001**	1.8	0.273	**3.7**	**0.001**	-1.9	0.226	**-2.5**	**0.047**	1.2	0.623
2.215	0.1	1.000	0.6	0.97	**4.2**	**<0.001**	1.8	0.285	**4.8**	**<0.001**	-0.8	0.841	2.1	0.122
3.765	**-5.1**	**<0.001**	1.2	0.67	2.1	0.147	**3.2**	**0.007**	**5.5**	**<0.001**	1.3	0.487	-1.4	0.492
6.4	0.5	0.982	-1.5	0.48	1.9	0.224	-0.5	0.981	**4.1**	**<0.001**	–	–	0.9	0.832

Notes: Significant differences are marked with bold. Abbreviations of the generations can be seen in Table 1.

except in 1.3 ml L^{-1}, was higher than the control (Supplementary Material, Table S2). The final length, absolute growth, the total number of eggs, number of clutches, the ratio of egg diameters, egg volume, reproduction investment, food consumption, and movement energy were not significantly different from the control group (Supplementary Material, Table 2).

In the F1 transgenerational treatment, the variance of the egg volume at 2.2 ml L^{-1} (marginally significant), the movement energy at 2.2 ml L^{-1}, and the trade-off at 0.77 and 2.2 ml L^{-1} concentration was significantly lower than the control. The variance of the size of the first clutch was higher in all treated groups comparing to the control (Table 2). The variance of the time of maturation at 3.8 ml L^{-1}, and the unhatching ratio at all except at 2.2 ml L^{-1} concentration was higher than that of the control (Supplementary Material, Table S3). The initial length, final length, absolute growth, the total number of eggs, number of clutches, the ratio of egg diameters, egg volume, reproduction investment, food consumption, and velocity were not significantly different from the control group (Supplementary Material, Table 3).

3.3. F2 generation

In the F2 multigeneration treatment, the variance of the total number of eggs at 2.2 ml L^{-1}, the velocity at 1.3 ml L^{-1}, and the trade-off at 0.77, 1.3 and 3.8 ml L^{-1} (Table 3) concentration was lower than that of the control. The variance of the initial length at 0.77 ml L^{-1}:concentration was higher when compared to the control. The variance of the size of the first clutch was marginally higher at 0.77 ml L^{-1} concentration but lower in every other case (Table 2). The final length, absolute growth, number of clutches, time of maturation, the ratio of egg diameters, egg volume, unhatching ratio, reproduction investment, food consumption, and movement energy were not significantly different from the control group (Supplementary Material, Table S4).

In the F2 transgenerational treatment, the variance of the total number of eggs at 3.8 ml L^{-1}, the size of the first clutch at 2.2 ml L^{-1}, and the egg volume at 3.8 ml L^{-1} concentration were lower than at the control group. The variance of the speed velocity was at the 0.77 ml L^{-1} (marginally), and the trade-off in the three highest concentrations (Table 3) was lower than that of the control. Initial length, final length, absolute growth, number of clutches, time of maturation, the ratio of egg diameters, unhatching ratio, reproduction investment, food consumption, velocity, and movement energy were not significantly different from the control group (Supplementary Material, Table S5).

3.4. F3 generation

In the F3 multigenerational treatment, the variance of the size of the first clutch in two concentrations (Table 2) and of the food consumption at 0.77 ml L^{-1} was lower when compared to the control. The variance of the trade-off was higher at 0.77 ml L^{-1} and lower at 1.3 ml L^{-1} concentration than that of the control (Table 3). The initial length, final length, absolute growth, the total number of eggs, number of clutches, time of maturation, the ratio of egg diameters, egg volume, unhatching ratio, reproduction investment, velocity, and movement energy were not significantly different from the control group (Supplementary Material, Table S6).

In the F3 transgenerational treatment, the variance of the initial length at 0.77 and 2.2 ml L^{-1}, the absolute growth at 0.77 ml L^{-1}, and the number of clutches at 6.4 ml L^{-1} (marginally) concentration were lower than that of the control. The size of the first clutch had a lower variance compared to the control in two cases (Table 2). The total number of eggs, time of maturation, the ratio of egg diameters, egg volume, unhatching ratio, reproduction investment, food consumption, velocity, movement energy, and trade-off were not significantly different from the control group (Supplementary Material, Table S7).

4. Discussion

A statistically significant difference in the variances from the control was found in a relatively high ratio (about one third) of all possible cases. This finding supports the hypothesis that the variance alteration in a dose-response study is an unexploited source of useful information about the effects of the toxicants (Bennett, 1987; Calow, 1996; Nikinmaa and Anttila, 2019). However, the results were mostly idiosyncratic. The variance of most parameters differed significantly from the control in just one or some concentration groups of different generations and treatments.

Only the variance of two parameters out of the fifteen (variance of the size of the first clutch and trade-off) showed a consistent pattern over

the generations in both treatments. This finding is in agreement with the statement of Walsh and Blows (2009), who concluded that variance changes appear only in a few parameters.

The first clutch size variance was significantly different from the control in all treated groups, which may be the result of the trait flexibility of the first clutch size in *F. candida* (Tully and Ferrière, 2008). The first clutch size is bound up with the body size, maturation and growth rate of this species (Stam et al., 1996). Insecticide exposure commonly enhances parameter variability in the short term (Forbes and Depledge, 1996; Orlando and Guillette, 2001). Orlando and Guillette (2001) argue that the increase of variance could be the first step in the evolution of the population in a changing environment. Variable offspring may have a better chance to survive in a heterogeneous, unpredictable environment in the short-term (Otto, 2008; Stearns, 1993). The first clutch size variances of all pesticide-treated groups were significantly higher than that of the control in the P and F1 generations, both in the multi- and transgenerational treatments. This means that there is a strong and unambiguous insecticide effect on F1 generation variance of this parameter which is in accordance with theoretical expectations of Forbes and Depledge (1996) and Orlando and Guillette (2001). The increased variance can be a maternal effect in reaction to the changing environment, while the offspring population with increased variance can survive with a higher chance in a heterogeneous environment (Coutellec and Barata, 2011; Orlando and Guillette, 2001).

Further explanation of the enhanced variance could be that the main force shaping the variance pattern was a disruptive selection of epigenotypes. If so, two strategies could emerge for the *F. candida* individuals in the first generation. First, some individuals do not invest much energy during the first clutch, rather into detoxification. This situation is comparable to the classical trade-off of *Drosophila melanogaster*, when low early fecundity is associated with stress-resistance and longevity (Zera and Harshman, 2001). Similarly, when mosquitoes build up an organophosphate resistance through an extensive expression of the detoxifying esterases, the development time and fertility is decreasing (van Straalen and Timmermans, 2002). Second, other individuals invest more energy in the first clutch. In this case, although the individuals died early, they still had the same reproductive output as the strategist before. Collembolans following the first strategy decrease the first clutch size, while those following the second strategy increase it. If both strategies are advantageous in the presence of the pesticide, then the mechanism behind the variance increase of the first clutch size is disruptive selection of epigenotypes. However, this could be a low probability explanation, while F2-F3 generation follows a different pattern because disruptive selection increases variance through more than one generations (Roff, 1997). Another low probability scenario is that the mutation rate increases due to xenobiotic stress (van Straalen and Timmermans, 2002), and therefore the variation of the parameter increases as well.

Similar responses of the first generation both in the multi- and transgenerational treatments can be a response of the insecticide's effect on the eggs of the parent generation. A well-known fact is that the F1 generation may be affected during the embryonic development within the exposed body of a mother (Shaw et al., 2017; Youngson and Whitelaw, 2008). That is why a similar response (increased variance compared to the control) both in multi- and transgenerational F1 offspring is not surprising. However, in the F2 and F3 generations, the variance in the first clutch size is lower than in the control cases, where a significant difference occurred (in half of the possible cases). Kingsolver et al. (2001) demonstrated that in the short term (days to a month, like P and F1 generation in our case), strong selection acts on viability parameters. In the long-term, however, non-viability parameters (like reproduction parameters) go through a stronger selection (F2 and F3 generation in our case). While the first clutch is a reproduction parameter and we did not find changes in the mean, though the variance decreased, the mechanism in F2-F3 generations could possibly have been a stabilising selection of epigenotypes.

The variance changes of the trade-off did not show such an unambiguous pattern as the first clutch size, but the mean of the trade-off changed over-generations especially in the multigenerational line (Szabó et al., 2020). The trade-off is the resultant of several other parameters (diverse reproduction and growth parameters, but food consumption may also have an influence). One can hypothesise that these changes of variance are a consequence of the interrelationship of the first clutch size and trade-off (Chapman, 2001) because the first clutch size gives a part of the total egg number. Reproduction-growth trade-offs are very flexible (Zera and Harshman, 2001). According to Kingsolver and Diamond (2011), more explanations are plausible. The environmental changes (in our study, this was the insecticide treatment) can mildly affect several parameters in different directions, which makes measuring selection in trade-offs difficult. Moreover, according to Kingsolver and Diamond (2011), it is possible to detect fluctuations in the selection, which could make the parameter changes slighter if the magnitude of the selection is high. However, in this case, the long-term effects have to show the same direction. In our case, the variance of the trade-off decreases in most cases, so the latter is a possible scenario.

Another possible reason for the inconsistencies in the variance changes in the trade-off is that selection of epigenotypes on growth can be easily impaired by indirect effects (Kingsolver and Diamond, 2011), which is a substantial part of our trade-off. We hypothesised that a less unambiguous pattern of the trade-off variance than that of the first clutch size could be observed because all parameters involved in the trade-off (absolute growth, total number of eggs (which include the size of the first clutch, and mean egg volume) are variable. Their variance and other statistical characteristics can sometimes even oppositely change. Furthermore, trade-offs between parameters can have a time-lag, which makes the interpretation even more challenging (Zera and Harshman, 2001).

A clear-cut difference between mean and variance reaction to insecticide treatments was observed at the first clutch size. The mean of this parameter did not show a difference between the control and treated collembolan groups. The selection of epigenotypes may not have affected the mean of the parameter because it was near to the fitness peak, and that is why the directional selection effect on the mean was weak (Walsh and Blows, 2009).

Nikinmaa and Anttila (2019) enumerate three primary sources of the individual variation as (i) experimental circumstances, (ii) genetic variation, and (iii) genotype independent phenotypic plasticity. The effects of the first two sources were kept as low as possible in this study. Consequently, genotype independent phenotypic plasticity, which can be caused by developmental plasticity (possibly maternal effects) and/or epigenetic mechanisms, should be the main reason for variance changes in our study. This hypothesis is supported by the fact that the Trebon 30 EC evokes epigenetic events in *F. candida* (Szabó et al., 2019a, 2020).

As for the generality of our results, there are some examples for variance increase due to toxicant application at short-term on different sexual species (this study, Forbes and Depledge, 1996; Holloway et al., 1997; Orlando and Guillette, 2001, Hoffmann and Parsons, 1997; Sgrò and Hoffmann, 1998). Therefore, it seems plausible that the short-term variance increase due to toxicant exposure is a general phenomenon in ecotoxicology. We do not know other experiments what tested the variance changes in such a long-term as we did. Although, Nikinmaa et al. (2019) had found variance decrease in the case of *D. magna* in parent generation but not in F1 and F2 generations. Besides, Orlando and Guillette (2001) stressed out that variance decrease is a possible reaction to increased stress. We found a variance decrease in the later generations (F2 and F3). This result could be explained in the multigeneration treatment in our study with stabilising selection, when intensive stress, consequently strong selection pressure threat the animals. So, the situation becomes similar to the intense treatment of Nikinmaa et al. (2019). However, this similarity does not apply to the transgenerational treatment, while the stress does not increase in this line but decreases. However, to test the generality of variance decrease

in multigeneration ecotoxicological studies needs further studies.

According to Smit et al. (2001) inter-individual variance changes are useful as extra information for statistical modelling and modelling sensitivity of the species. However, using the variance in modelling sensitivity of the species is rather a technical use than practical. In our experiment, the variance was more sensitive to treatment than the mean of the first clutch size. Therefore, variance changes could be an early sign of stress in some cases. Moreover, variance changes are easy to test and have no additional costs.

We have to point out that the change of variance in F1 compared to the F2 and F3 generations in the opposite direction has a central importance in the environmental risk assessment (ERA). This result of our study confirms the opinion that ERA should be based on multigenerational studies as soon as possible (Breitholtz et al., 2006).

5. Conclusions

Our data support the view that besides the mean, a statistical analysis of the variability of the parameters could be a considerable measure of environmental disturbance in the form of insecticide application if the suitable parameter is found. Consequently, change of the variance is "not only unwanted noise" but a possible source of new information in ecotoxicology. Indeed, an enhanced variance is often observable in exposed populations as an early sign of the disturbance. We need to emphasise that in our study, variance changes were already indicative at sublethal insecticide concentrations. Many scientists argued that the variance could be a piece of extra information in ecotoxicology. However, testing variance changes are important because variance change can signalise toxicant impact even when the mean does not change in certain cases.

Vast amounts of ecotoxicological dose-response studies are available in which inter-individual parameter variance was not analysed in detail as in our work. Therefore, we suggest that additional studies need to be conducted to exploit these sources of information further and find suitable parameters of various species, which show variance response to toxic influence. We have to point out that for each species and pesticide group, the appropriate parameters for analysis have to be found.

Supplementary material

Table S1–S7 contains the full statistical analysis of all parameters in every generation. The mean data per animal is given as Mendeley data (Szabó et al., 2019b).

CRediT authorship contribution statement

Borbála Szabó: Conceptualization, Methodology, Investigation, Formal analysis and Writing. **Zsolt Lang**: Formal analysis and Validation. **Szilvia Kövér**: Validation and Writing. **Gábor Bakonyi**: Conceptualization, Methodology, Investigation and Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112260.

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