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Red Flour Beetles as a Model System to Study Transgenerational Effects of a Psychoactive Drug

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Abstract

Pharmaceutical testing on epigenetic effects are very limited. The routine use of vertebrates as preclinical research models is constrained by their long mating and generational times, leading to very low numbers of progeny to test and assess. In contrast, invertebrates such as insects are ethically acceptable, and convenient as a laboratory model because they breed rapidly, and produce plenty of offspring under the right circumstances. Here, we used the red flour beetle, *Tribolium castaneum*, as a model to screen for the effect of drugs on behavioral traits. We tested environment/diets supplemented with the experimental drug, valproic acid (VPA), which is a histone deacetylase inhibitor, at different concentrations (0.01%, 0.1%, 1%) and a control concentration with 0% VPA. VPA is a commonly prescribed psychoactive drug used in the treatment of migraines, epilepsy etc. VPA is known as a teratogen. The aim of this study was to examine effects on behavior, fecundity, viability, and survival from exposure to this drug. We found no difference in egg production or viability in parental generation. Further, F_1 survival was not different among the beetles from different environmental concentrations of VPA.

Keywords: *Tribolium casteneum*, valproic acid, transgenerational effects

1. Introduction

Epigenetics refers to processes that influence gene activity but not the genetic sequence[11]. Studies show that epigenetic mechanisms can lead to modifications that can be transmitted to daughter cells[11]. Chemicals such as in pharmaceuticals, supplements, environmental contaminants etc. can have transgenerational effects on organisms[1-6, 10]. A wide range of transgenerational effects of chemicals have been documented in animals. Recent works suggest that transgenerational effects of pharmaceuticals may include heart disease, cancer, neurological and cognitive disorders, obesity, diabetes, infertility, and sexual dysfunction in humans[5].

Pharmaceutical testing on epigenetic effects are very limited[2]. The routine use of vertebrates as preclinical research models is constrained by their long mating and generational times, leading to very low numbers of progeny to test and assess. In contrast, invertebrates such as insects are ethically acceptable and convenient as a laboratory model because they breed rapidly, and produce plenty of offspring under the right circumstances. The red flour beetle, *Tribolium castaneum*, has all the above characteristics and suitable as a model sytsem[8]. Here, we used *T. castaneum*, to screen for the effect of a pharmaceutical on fitness traits.

We tested environment/diets supplemented with the experimental drug, valproic acid (VPA), which is a histone deacetylase inhibitor[7]. This drug is an anticonvulsant and used to treat seizures, mania and migraine[7]. Thus, VPA is commonly prescribed in the treatment of migraines, epilepsy etc[7]. VPA is known as a teratogen[5,7]. Beetles were exposed to VPA at different concentrations (0.01%, 0.1%, 1%)[2]. We hypothesize that exposure to VPA will alter

behaviors and result in the decline of fecundity and fertility in the parent and F_1 generation. We predicted that: 1) Beetles placed in VPA medium will have lower egg production compared to controls (plain flour), 2) Eggs placed in VPA medium will have lower survival to larvae and subsequent stages compared to eggs placed in the controls (plain flour) and 3) Eggs placed in VPA medium will exhibit more activity as larvae.

2. Methods and Materials

2.1. Beetle Source and Maintenance

Carolina strain beetles were acquired from Carolina Biological Company. Beetles were raised in standard flour and yeast medium (95% flour, 5% yeast) in a dark incubator kept at 29ºC at 70% RH[9].

2.2. Collecting eggs

We mated adult beetles from a stock jar in finely sifted flour for 5 days. Eggs were collected from the jars and counted for use in the experiments.

2.3. Creating environments with different concentrations of VPA

Finely sifted flour was mixed with VPA in three different concentrations. Three jars each at 0.01%, 0.1%, or 1% VPA (w/w) were set up. This was done by creating mediums with 99g of flour and 1g VPA, 99.9g of flour and 0.1g of VPA or 99.99g of flour and 0.01g of VPA[2]. Control jars had no VPA.

2.4. Experiment 1: Measuring production of eggs

Pupae were extracted from stock jars. Males and females were separated and kept in groups of 5 till approximately 7 days post emergence. Five adults of each sex were placed in environments with different concentrations of VPA. Egg production was measured every 4-6 days. Once counted, eggs were placed back in the jar. Thus egg/larvae counts were cumulative.

2.5. Experiment 2: Measuring survival of eggs

We placed 50 eggs in each of the above environments. Every 5^{th} day, larval counts were taken for each jar. In this manner, we determined how many eggs survived to the adult stage of their life cycle.

2.6. Experiment 3: Measuring survival of F_1

Once eggs from Experiment 1 developed to adulthood, adults were allowed to mate in finely sifted flour. To avoid inbreeding, adults from the three replicates within each concentration of VPA were mixed at random to create the next generation. Once adults had mated, we collected eggs from them as described in 2.2. Then 10 eggs were placed in finely sifted flour with no VPA to test for transgenerational effects of VPA.

2.7. Statistical Tests

Fitness traits including 1) egg production, 2) egg survival, and 3) F_1 survival for control and VPA environment beetles were compared using Analyses of Variance or Survival analysis. Because egg production data were cumulative, only week 4 data were used to compare the egg production of beetles in different conditions of VPA. Egg and F_1 survival was compared using survival analysis.

3. Results

3.1. Experiment 1: Measuring production of eggs

All jars had eggs, thus beetles in all environments produced offspring successfully. On average the control group had 48.66 (SD 9.56) eggs whereas the 1% VPA environment beetles produced 37.66 (SD 4.16) (Figure 1). Egg production did not differ in the different environments (ANOVA, df = 3, error = 8, F = 1.24, P = 0.34).

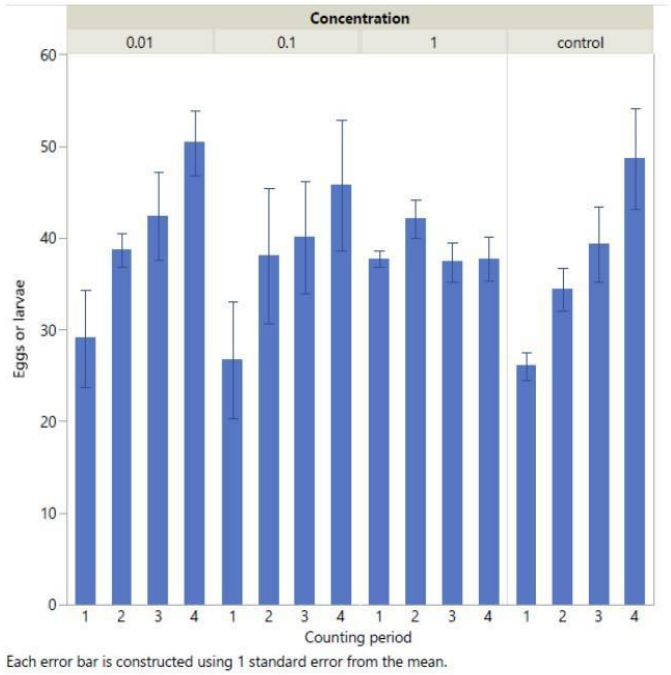

Figure 1: Egg production was not affected by VPA in the environment (ANOVA, P = 0.34).

3.2. Experiment 2: Measuring survival of eggs

Survival of eggs was high in all treatments for the first 5 weeks. Starting with approximately 50 eggs in each jar, the numbers of eggs surviving into the 5th week were as high as 44 to 47 (Figure 2), however survival declined in the 6th and 7th week. By the 7th week the numbers of eggs surviving were on average 23 (0.1% VPA) to 33 (in 1% VPA) (Figure 2). Overall, a large number of eggs survived to adulthood (Figure 2). Survival of eggs did not differ significantly among the treatments (Survival analysis, df = 3, Chi- square = 1.33, P = 0.72).

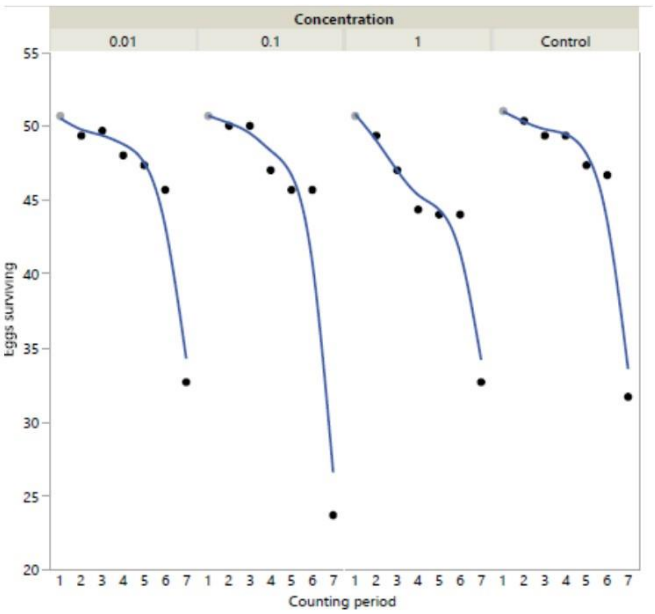

Figure 2: Survival of eggs did not differ among the environments with different concentrations of VPA (P = 0.72).

3.3. Experiment 3: Measuring survival of F_1

F_1 survival was very high, almost 100%, in all conditions (Figure 3). In most cases all 10 F_1 progeny survived during the experimental period. Survival was not different among the different concentrations of VPA (Survival analysis, df = 3, Chi- square = 1.18, P = 0.75).

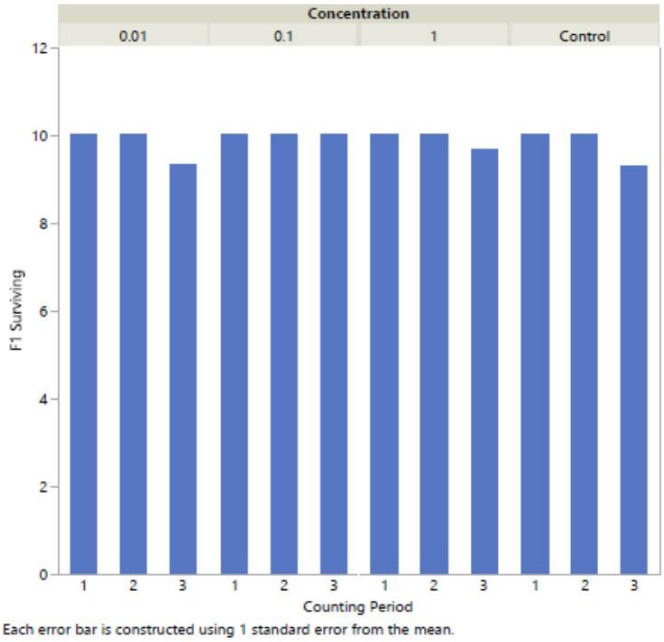

Figure 3: F_1 survival did not differ among the environments with different concentrations of VPA (P = 0.75).

4. Discussion

Recent studies have shown that pharmaceuticals and other chemicals have transgenerational effects on the organisms exposed to them[1-6,10]. Transgenerational effects of environmental chemicals are known for invertebrates as well vertebrates[1-6,10]. In a medaka fish, exposures of parent fish to either BPA (bisphenol A) or EE2 (17a ethinylestradiol), two environmental contaminants, during early stages of development, resulted in reduction in the fertilization rate in F_2 offspring and embryo survival in F_3 generation[1] .

The commonly used chemical tributyltin (TBT, an anti-fouling agent) is known to be an obesogen, and babies with higher exposure to this chemical have higher corpulence index[6]. This chemical is thought to have transgenerational effects on obesity by reprograming stem cells[6]. Other chemicals such as triphenyltin (TPT, a miticide) and dibutyltin (DBT, a heat stabilizer) are thought to affect obesity in a similar way[6]. Similarly, endocrine disrupting chemicals such as polychlorinated biphenyls and dioxins are also thought to have negative transgenerational effects on both male and female reproduction[3].

The common pharmaceutical, VPA is associated with altered gene expression and skeletal malformation[5]. In the mouse model system, exposure to VPA is known to induce autism like behaviors in F_2 and F_3 generations[4]. In a previous study on the red flour beetle, egg production as well as survival declined due to transgenerational effects of VPA[2]. The previous study demonstrated that in the 0.1% VPA condition, egg development showed no signs of impairment, but at the higher concentration of 1% VPA, the development was slower and mortality was higher compared to that in the control environment [2]. However, our study could not replicate this result, most probably due to the shorter time frame (F_1 survival was measured over only 2 weeks) or genetic differences in beetle strains used in the two studies. In our study, we found that there were no differences in survival among the different environment concentrations in both eggs and the F_1 generation.

The spate of studies detailing transgenerational effects on health of humans as well as other species underscore the importance of developing model systems in which transgenerational effects can be studied quickly and relatively inexpensively[1-6,10-11]. The red flour beetle has been used a model system for genomics, evolution, behavioral ecology[8] and can be used as a model system for epigenetics. Future work will attempt to replicate this study for a longer time period.

5. Acknowledgements

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