Stamenković-Radak, M., G. Rasić, M. Čučković, T. Savić, and M. Andjelković 2008, Folia biol-Kraków 56: 51-56; Terzić, T., M. Anđelković, and M. Stamenković-Radak 1994, Dros. Inf. Serv. 75: 164-165; Terzić, T., M. Andjelković, D. Marinković, and M. Stamenković-Radak 1996, J. Evol. Biol. 9: 337-355; Zar, H.J., 1984, *Biostatistical Analysis*, 2nd ed., Englewood Cliffs, New Jersey: Prentice Hall Inc.

Effects of fungicide Dithane M 45 in *Drosophila melanogaster* on courtship behavior.

Vasudev, V.[1,*], H.P. Gurushankara[2], P. Vishwaprakash Mahadimane[1], D. Khalandar[1], and B.R. Shamprasad[1]. [1]Department of Studies in Bioscience, University of Mysore, P.G. Center, Hemagangothri, Hassan-573220, Karnataka, India; [2]Department of Animal Sciences, School of Biological Sciences, Central University of Kerala, Kasaragod, Kerala-671328, India. *To whom correspondence should be addressed; Email: profvvasudev@gmail.com

Introduction

The dithiocarbamate pesticide Dithane M 45 is being used extensively to kill fungi. Although its primary function is to increase crop yield and food production, researchers showed that it has toxic effects and effect on genetic recombination in *Drosophila melanogaster* (Vasudev and Krishnamurthy, 1976, 1979, 1982), chromotoxic effects in plants (Pandey *et al.*, 1994), toxic effects on alga, *Stichococcus bacillaris* (Marton, 1974), embryo mortality in chickens (Keseru *et al.*, 2003), and non-clastogenic in mice (Vasudev and Krishnamurthy, 1994).

D. melanogaster has been proved beyond doubt as the best available sub-mammalian test system to screen genotoxic effects of environmental pollutants (Vogel and Sobels 1976; Sobels 1974; Vasudev, 1980; Wurgler *et al.*, 1985; Siddique *et al.*, 2005). Even though protocols of *D. melanogaster* have been validated for genotoxicity studies, time and again attempts are being made to introduce inexpensive, short duration and efficient parameters. Hence, in this direction, we presume that courtship behavior may be used as a parameter to understand the effects of environmental pollutants. It is pertinent to mention here that *D. melanogaster* with its well established series of sequential stereotyped elements of courtship behavior (Spieth, 1974, 1983; Bastock and Manning, 1955; Guruprasad *et al.*, 2010), an attempt has been made to use this protocol to understand the genetic effects of environmental pollutants. Nonetheless, Yamamoto and Koganezawa (2013), Dauwalder (2011), and Latham *et al.* (2013) have demonstrated that *fruitless* and *doublesex* genes are involved in courtship behavior. Furthermore, until now as far as we are aware there are no reports on the effects of environmental pollutants on courtship behavior. Therefore, the present work has been undertaken to understand the effects of a fungicide Dithane M 45 on courtship behavior of *D. melanogaster* and to authenticate this protocol for genotoxicity studies.

Materials and Methods

Dithane M 45, a zinc ion manganese ethylene bisdithiocarbamate, where 2% zinc, 16% manganese, and 62% ethylene bisdithiocarbamate obtained from Indofil chemicals Ltd, Mumbai, India and *D. melanogaster* Oregon-K strain were used for the present studies.

Wheat cream agar medium containing sub-lethal concentrations of Dithane M 45 (50, 100, and 150 ppm) were prepared and distributed to food vials (Vasudev and Krishnamurthy, 1979). Normal medium was used as control. 25 eggs per vial were collected following Delcour (1969) technique, and newly-hatched larvae were continuously fed on the above food media, *i.e*., larval feeding method was used. When adults emerged, virgin females and bachelors were isolated within four hours of eclosion and maintained separately in normal media for five days.

The following combination of crosses were made for observing the effect of Dithane M 45 on courtship behavior:

(A) Control crosses (untreated males × untreated females)

(B) Male treated crosses (treated males × untreated females)

(C) Female treated crosses (treated females × untreated males)

(D) Both treated crosses (treated males × treated females)

Single virgin female was aspirated out gently and introduced into an Elens-Wattiaux mating chamber (Elens and Wattiaux, 1964). A bachelor male was added to it and allowed to acclimatize to the chamber for 30 seconds. The details of courtship behavior were directly observed through hand lens of 10× magnification. All the experiments were conducted during morning (7-10 A.M.) in a room with a temperature of 24 ± 1˚C under normal laboratory light condition. Orientation, tapping, wing vibration, licking, and copulation duration were recorded separately and simultaneously by two observers in the control and treated group. From these data, courtship latency and copulation latency were analyzed and tabulated. Means and standard errors were calculated. Two-way ANOVA using DMRT statistical test was carried out.

Results and Discussion

In both treated and control groups, it has been observed that male engages in a series of actions, which include orientation towards females, tapping her with his fore legs, courtship song by expanding and vibrating his wings, licking the female's genitalia, curling his abdomen to attempt copulation, and lastly mounting the female by holding with first forelegs. Thus, in the present study, qualitative data are similar to that described by Bastock and Manning (1955) and Spieth (1974). However, the results in Tables 1, 2, and 3 revealed that there are quantitative differences between control and different treated groups. It is clear from these tables that courtship elements such as orientation, tapping, wing vibration, and licking are significantly increased in dose dependent manner. Further, values of higher doses (100 and 150 ppm) of different groups are highly significant compared to control ($p < 0.05$). Thus, higher doses of Dithane M 45 are effective in altering courtship behavior of *D. melanogaster*. Nontheless, Dithane M 45 induced toxicity in the form of increased rate of development and reduced viability (Vasudev and Krishnamurthy, 1979). It has also been demonstrated that Dithane M 45 significantly reduced the morphological characters such as body length, wing length, and size of the pupae above 50 ppm compared to controls. Hegde and Krishna (1999) showed that bigger the better in courtship behavior of *D. malerkotliana.* Thus, it can forward the opinion that reduced body size and wing size of *D. melanogaster* due to Dithane M 45 results in altered courtship behavior, which is a disadvantage for the flies.

Courtship latency is the time lag before performance of the first courtship behavior (orientation) after pairing (Ejima and Griffith, 2007) or it is the period during which the pairs acclimatize in the mating chamber and then start courtship activities. It actually indicates the vigor of the male (Markow, 1985). The courtship latency is shown to be significantly longer in treated groups

($p < 0.05$, Tables 1, 2, and 3) compared to control. Thus, it can be said that males require longer duration to attract the females.

Table1. Effect of Dithane M 45 on courtship elements of *D. melanogaster* male.

Behavioral elements	Control	50 ppm	100 ppm*	150 ppm*
Orientation	25.4 ± 0.72	26.4 ± 0.76	32.7 ± 0.65	42.03 ± 0.66
Tapping	3.7 ± 0.56	4.01 ± 0.60	5.2 ± 0.59	7.02 ± 0.62
Wing vibration	4.6 ± 0.4	5.2 ± 0.49	7.06 ± 0.84	12.07 ± 0.65
Licking	2.9 ± 0.07	3.01 ± 0.48	5.01 ± 0.36	8.95 ± 0.50
Courtship latency (sec)	10.9 ± 0.32	15.7 ± 0.72*	17.8 ± 0.75	19.6 ± 0.78
Copulation latency (mins)	9.1 ± 0.89	12.3 ± 0.25*	14.5 ± 0.67	22.5 ± 0.49
Copulation duration (mins)	16.3 ± 0.87	13.4 ± 0.28*	14.2 ± 0.36	13.3 ± 0.61

*P < 0.05

Table 2. Effect of Dithane M 45 on courtship elements of *D. melanogaster* female.

Behavioral elements	Control	50 ppm	100 ppm*	150 ppm*
Orientation	25.4 ± 0.72	27.4 ± 0.60	34.6 ± 0.59	45.3 ± 0.66
Tapping	3.7 ± 0.56	4.75 ± 0.40	6.2 ± 0.48	8.1 ± 0.78
Wing vibration	4.6 ± 0.4	5.75 ± 0.72	8.01 ± 0.36	13.07 ± 0.65
Licking	2.9 ± 0.07	3.25 ± 0.21	6.02 ± 0.18	9.25 ± 0.11
Courtship latency (sec)	10.9 ± 0.32	12.25 ± 0.23	18.02 ± 0.19	20.1 ± 0.25
Copulation latency (mins)	9.1 ± 0.89	13.02 ± 0.40*	15.03 ± 0.39	24.75 ± 0.28
Copulation duration (mins)	16.3 ± 0.87	14.01 ± 0.68*	13.2 ± 0.61	14.2 ± 0.62

*P < 0.05

Table 3. Effect of Dithane M 45 on courtship elements of *D. melanogaster* in both male and female.

Behavioral elements	Control	50 ppm	100 ppm*	150 ppm*
Orientation	25.4 ± 0.72	26.75 ± 0.05	34.15 ± 0.08	44.15 ± 0.12
Tapping	3.7± 0.56	4.25 ± 0.15	5.75 ± 0.17	8.25 ± 0.18
Wing vibration	4.6 ± 0.4	5.75 ± 0.05	8.01 ± 0.12	15.15 ± 0.21
Licking	2.9 ± 0.07	3.25 ± 0.03	6.02 ± 0.14	9.25 ± 0.18
Courtship latency (sec)	10.9 ± 0.32	16.25 ± 0.06*	18.75 ± 0.18	22.85 ± 0.22
Copulation latency (mins)	9.1 ± 0.89	13.25 ± 0.09*	15.2 ± 0.29	24.0 ± 0.26
Copulation duration (mins)	16.3 ± 0.87	12.0 ± 0.16*	14.0 ± 0.21	12.75 ± 0.28

*P < 0.05

Copulation latency is measured as the time taken for the male to orient towards female until initiation of copulation (Markow, 1985) or it is the time lag before successful mounting after pairing (Ejima and Griffith, 2007). It actually indicates the vigor of male (Hegde and Krishnamurthy, 1979; Mansoor and Hedge, 2006). A male with high vigor reacts quickly in the presence of a female, whereas a male with less vigor reacts slowly. On the other hand, it also reflects the receptivity of females. Obviously, longer copulation latency indicates lesser vigor of males and also non-receptivity of females. The longer copulation latency was notified in experimental treated groups suggesting that the females are non-receptive and males are less vigorous. Thus, this element not

only indicates the vigor of males but also receptivity of females. A male with high vigor has to perform the same courtship act many times to a non-receptive female than to a receptive female and vice versa. It is clear from the results that the treated males could not maintain high vigor and treated females are less receptive.

Courtship activity of the male or female culminates in copulation as suggested by Spiess (1970). During copulation, sperm from the male are transferred to the female reproductive tract and, therefore, the duration of copulation has a lot of significance in an animal's life. According to Guruprasad *et al.* (2008) longer duration of copulation permits the transfer of more sperm by males, thus enhancing the fitness of males. It also enhances the fitness of the females, because the sperm received by a female can fertilize more eggs. In the present study in the treated groups, the copulation duration is significantly less compare to control. This proves that both treated male and treated female are less fit compared to non-treated ones (Table 3). On par with this, treated flies are less fecund than controls (Vasudev and Krishnamurthy, 1982). It is also demonstrated that mean daily egg production is reduced with reduced size of imago (Vasudev and Krishnamurthy, 1982). When the data are pooled together it is interesting to note the toxicity in terms of less viability, less fecund and reduced size of imago on one hand and effect on courtship behavior leading to less vigor and less receptivity on the other. All these factors are coinciding, resulting in the effect of Dithane M 45 on fitness of *D. melanogaster*.

Conclusion

The advantages of *D. melanogaster* to be used as a model organism in courtship behavior are: 1) they are small but not minute organisms (2.7mm); 2) short adult life span; 3) sexual dimorphism; 4) single pair mating can be easily achieved; 5) elaborate courtship behaviors; 6) known pedigree can be studied; 7) courtship behaviors can be analyzed qualitatively and quantitatively. These are similar under natural and field conditions and 8) many behavioral mutants are available. Hence, it is the strong opinion of the authors that *D. melanogaster* is an appropriate model to test the effects of sub-lethal concentrations of environmental pollutants using different courtship activities within short period of time.

References: Bastock, M., 1956, Evolution 34: 421-439; Bastock, M., and A. Manning 1955, Behavior 8: 85-110; Dauwalder, B., 2011, Int. Rev. Neurobiol. 99: 87-105; Delcour, J., 1969, Dros. Inf. Serv. 44: 133-134; Ejima, A., and L.C. Griffith 2007, Cold Spring Harbor protocols. p4847; Elens, A.A., and J.M. Wattiaux 1964, Dros. Inf. Serv. 39: 118; Guruprasad, B.R., S.N. Hegde, and M.S. Krishna 2008, Zoological Studies 47: 75-83; Guruprasad, B.R., S.N. Hegde, and M.S. Krishna 2010, Journal of Insect Science 10: 123; Hegde, S.N., and M.S. Krishna 1999, Curr. Sci. 77: 178-179; Hegde, S.N., and N.B. Krishnamurthy 1979, Australian Journal of Zoology 27: 421-431; Keseru, M., S. Fejes, P. Budai, and L. Varnagy 2003, Commun. Agric. Appl. Biol. Sci. 68: 813-815; Latham, K.L., Y.S. Liu, and B.J. Taylor 2013, BMC Neuroscience 14: 57; Mansoor, N., and S.N. Hegde 2006, J. Pakistan Med. 90: 58-63; Markow, T.A., 1985, Anim. Behav. 33: 775-781; Marton, A., 1974, Biol. Abst. 57: 34078; Pandey, R.K., R. Shukla, and S.K. Datta 1994, Cytologia 59: 419-422; Siddique, H.R., S.C. Gupta, A. Dhawan, R.C. Murthy, D.K. Saxena, and D.K. Chowdhuri 2005, Environ. Mol. Mutagen. 46: 189-97; Sobels, F.H., 1974, Mutation Res. 26: 277-284; Spiess, E.B., 1970, New York: Appleton-Century-Crofts. pp. 315-379; Spieth, H.T., 1974, Annu. Rev. Entomol. 19: 383–406; Spieth, H.T., and J.M. Ringo 1983, Mating behavior and sexual isolation in *Drosophila.* In*: The Genetics and Biology of* Drosophila (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). pp. 223-284. Academic Press, New York; Vasudev, V., and N.B. Krishnamurthy 1976, Proc. Dunn Dobzh. Symp. Genet. 81-89; Vasudev, V., and N.B.

Krishnamurthy 1979, Experientia 35: 528-529; Vasudev, V., 1980, Effects of two carbamate pesticides on *Drosophila* and mice. Ph.D Thesis submitted to University of Mysore; Vasudev, V., and N.B. Krishnamurthy 1982, J. Mysore Univ. 29: 79-86; Vasudev, V., and N.B. Krishnamurthy 1994, Mutation Res. 323: 133-135; Vogel, E.W., and F.H. Sobels 1976, The functions of *Drosophila* in toxicology testing. In: *Chemical Mutagens. Principles and Methods for Their Detection.* (Hollander, A., ed.), Vol. 4, 93-142; Wurgler, F.E., U. Graf, H. Frei, and Juon 1985, Mutation Res. p. 326; Yamamoto, D., and M. Koganezawa 2013, Nature Reviews Neuroscience 14: 681-692.

Enriched nutrient diet shortens the developmental time –A transgenerational effect in *Drosophila sulfurigaster sulfurigaster.*

Neethu[1], B.K., Y. Ramesh Babu[2], and B.P. Harini[1*]. [1]Department of Zoology, Bangalore University, Bangalore-560 056, Karnataka, India; e-mail: bpharini@ yahoo.co.in; [2]Centre for Applied Genetics, Bangalore University, Bangalore-560 056, Karnataka, India.

Abstract

Developmental time has a great relevance to fitness in all organisms. We set out to investigate the effect of parental larval diet on offspring development time, with relatively low amounts of sugar as a carbohydrate source and different concentrations of protein to assess the role of macronutrient balance on developmental time in *Drosophila sulfurigaster sulfurigaster* species. In the current study, the influence of larval diet experienced during just one generation extends into the next generation. Offspring reared on high protein and relative sugar concentration underwent metamorphosis significantly faster compared to the offspring of adults from low protein diet relative to sugar diets. A transgenerational effect of parental diet on offspring was found. Developmental time recorded was shortest in the case of offspring when compared to parents fed with high protein diet.

Introduction

Deficiency or imbalance of fat, carbohydrate, or protein can affect characters such as growth and reproduction. Protein deficiency reduces fecundity and growth in *Drosophila melanogaster* (Wang *et al.*,1995), and in fruit-feeders protein is often limiting macronutrients (Markow *et al*., 2001). Many organisms face a challenge of meeting their optional nutritional requirement for somatic and reproductive growth under natural conditions (Raubenheimer *et al.*, 1991). During development, body tissues constantly require a specific quantity and proportion of nutrients in order to attain optimal growth and performance (Bauerfeind *et al.*, 2005). In contrast diet restriction on mild starvation can increase longevity as well as tolerance to stressors such as heat stress (Wenzel and Smith, 2006) demonstrating the complexity of organismal nutrient acquisition and utilization. A variety of factors may affect organismal stress tolerance. These include physiological as well as behavioral changes. The bulk of studies on physiological and evolutionary responses to nutrient deficiencies focus on reproduction and fecundity (Naya, 2007). On the other hand, parents may also respond to environmental cues in ways that enhance offspring performance under particular