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Role of Juvenile Environment in pre-adult development and Adult metabolites in *Drosophila melanogaster*

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Abstract

The purpose of the present study was to ascertain the effect of heavy metal (HM) supplemented diet on pre-adult development and adult macro molecules in fly populations. Interestingly, supplementation of supposedly essential HM, FeSo4, delayed development while non-essential HM CdCl2 did not effect pre-adult development time. The carbohydrate levels in the freshly emerging flies from HM supplemented diet were significantly lower, suggesting that the flies had used up higher levels of carbohydrates in combating the harmful effects of HM during the developing stages. The lipid levels in the developmentally stressed flies were marginally lower while in the developmentally non-stressed flies they were marginally higher in flies from HM supplemented diet. The protein levels were significantly higher in the flies from HM supplemented diet compared to those from SM diet. Taken together these results, suggest that the stress response mechanisms are perhaps evolutionarily conserved across most animal systems.

Keywords: Industrialization; Heavy metals (HM); oxidative stress; antioxidants.

2. Introduction

Advent of industrialization, intensive agricultural practices and anthropogenic activities have lead to increased pollution load in water bodies as well as the atmosphere. Heavy metals are one category of atmospheric pollutants whose levels have continued to increase over the years (Hendry *et. al.*, 1992; Patra *et al.*, 1994).

They get accumulated with ease in environment due to their bio persistence that interferes with the metabolic processes in varied organisms (Copat *et. al.,* 2012; Opfer *et. al.,* 2011; Farombi *et. al.,* 2007). Both essential and non essential heavy metals, depending on their nutritional and physiological importance can be toxic beyond a certain threshold range. Iron being an essential heavy metal, is indispensable for life (Aisen *et.al.,* 2001) and cadmium on the other hand is non essential heavy metal. The deleterious effects caused by each of them have been studied extensively (Papanikolaou *et. al.,* 2005; Satarug *et. al.,* 2000).

Previously biological stress has been defined as an environmental alteration resulting in decreased fitness (Koehn & Bayne, 1989; Sibly & Calow, 1989; Hoffman & Parsons, 1991). Metals induced toxicity causes oxidative damage via generation of ROS that damage proteins, lipids and nucleic acids. In response to alteration in environment, organisms have evolved adaptive physiological mechanisms which aids in increased survival (Djawdan *et. al.,* 1998; Harshman & Schmid, 1998; Rion & Kawecki, 2007). Nutrient acquisition and allocation are two important processes that influence life history of organisms (Rose and Bardley1988). Particularly in insect system acquisition of metabolic resources prior to entering the final metamorphosis stage during which the larval tissue is remodeled into adult structures plays a pivotal role in fitness. While allocation of resources is suggested to involves trade-offs between survival and reproduction related traits (Zera and Harshman 2001), and trade-off strategies are expected to change with change in environment,making it an important aspect of evolutionary biology. *Drosophila melanogaster* is an important model organism that can be gainfully employed in understanding the heavy metal induced stress (Akins *et.al.,* 1992) as it contains distinct resource acquisition and utilization phases. Resource acquisition in *D. melanogaster* occurs during the three larval stages of the pre-adult phase, while resource allocation towards survival and reproduction occurs in the adult phase. In this study we assessed the affect of heavy metals contaminated diet during the larval stages on energy budget of the adult flies. Specifically we have ascertained how the accumulation of proteins, carbohydrates and lipids are affected due to exposure of growing larvae to heavy metal contaminated diet.

3. Materials and Methods

3.1 *Fly populations*

Six *D. melanogaster* populations were used in the present study. Three of the six populations were control populations that were maintained on 3 week egg-to-egg discrete generation cycle, while the other three were derived from the control populations and were experiencing faster pre-adult development and extended adult lifespan. The control populations were designated as JB owing to their origin and they trace their ultimate ancestry to IV population (Ives, 1970). The detailed maintenance protocol for JB populations is described in detail by (Prasad et al. 2001). The derived populations are designated as FLJ (F-Faster developing, L-Late reproducing, J-JB derived) are described in detail by Rajamani *et al.* (2006). All the JB and FLJ populations were maintained in standard laboratory conditions of 25 ± 1 °C temperature, 70 ± 5% RH and 24:0 L:D cycle (SLC) on standard banana-jaggery media (SM) (Chandrashekara and Shakarad, 2011). At the time of initiating this study, the JB populations had pre-adult duration of 9 and half days (from egg till emergence of adult), while those from the FLJ had 7 and half days. On an average both the females and males of the JB populations were twice as heavy as FLJs and had an average lifespan of 30 days as against 45 days for the FLJs.

3.2 *Standardized Flies and Synchronized L1 collection*

Differing maintenance conditions can give rise to non-genetic parental effects. Both JB and FLJ populations were passed through one generation of common rearing conditions in order to minimize any non-genetic parental effects other than the intended heavy metal effects. Parental flies for generating assay flies were derived from running cultures. Forty vials from each population with approximately 50 eggs per 6ml SM were incubated at SLC till the emergence of all adults. All the adults from 40 vials per population were transferred to pre-labelled clean population cage and provided with fresh SM plate supplemented with yeast-acetic acid paste for about three days. These flies were termed as Standardized flies (SF). The yeast supplement is known to boost the egg production in the flies. On the 4th day post-emergence eggs were collected and allowed to hatch. The newly emerged larvae (L1) were transferred to vials containing 6ml of either SM or SM supplemented with a known heavy metal of definite concentration. Eighty larvae were transferred per vial, per treatment, per population. In order to obtain larvae of uniform age, the eggs from the SF were obtained in 1 hour laying windows for four successive hours by providing clear agar plates. The eggs laid during the first 2 one hour windows were discarded and only the eggs laid in the subsequent 2 one hour windows were used.

3.3 *Standardization of Heavy metal concentration*

Preliminary assays were conducted to ascertain the LD_{50} dose for $FeSO_4$ (essential) and $CdCl_2$ (non-essential) the two chosen heavy metals. The optimum concentration chosen for further assay were 13mM and 5 µM for $FeSO_4$ and $CdCl_2$ respectively.

3.4 *Pre-adult Developmental Time Assay*

Eighty synchronized L1 larvae collected from SF were dispended into vials containing 6ml of treatment medium. Ten such vials were set per treatment per population. Once the pupae turned black and developed red eye-spots, a vigil check was carried out at two hourly interval to check for emergence of adults. On noticing the first emerging fly, the subsequent checks were carried out at four hour interval and all emerging flies were sorted according to their gender under light CO_2 anaesthesia and stored at -80 °C for future biochemical assays.

3.5 *Adult Dry weight, Fluid and Lipid estimation Assays*

Freshly emerged flies were etherized, sorted according to gender, distributed into groups of 10 flies each and weighed to the nearest microgram using microbalance (Model No. CM11, Citizen) to obtain fresh weights (FW). Each group was transferred into pre-labelled clean dry vial and dried at 70°C for 36 hours, and weighed to obtain dry weight (DW). Subsequent to obtaining DW, the flies were transferred to pre-labelled 1.5 mL eppendorf tubes for de-fatting in di-ethyl ether following the methods of Zwaan and colleagues (1995) with few modifications. 1.3 mL of diethyl ether was added and tubes were rocked on a gel rocker (20 rpm) at RT with change of ether every 12 hourly for 36 hours. At the end of 36 hours, flies were removed and washed with ether, dried at 70°C for 2 hours, and weighed to obtain lipid-free dry weights. Fluid content was estimated by subtracting DW from FW, while lipid content was estimated by subtracting dry weight post-lipid extraction from DM. Five replicate batches per gender/treatment/population were set up.

3.6 *Carbohydrate Content*

Carbohydrate was assayed by Anthrone method (Van Handel 1965) with slight modifications. Briefly 10 flies were homogenized in O.1M PBS and then centrifuged for 12000 rpm at 4°C for 10 minutes. The crude supernatant was then diluted ten times.600μl of diluted supernatant was taken to which 5400μl of 95% Anthrone Reagent (BC0088 Biochem. Life Sciences) was added and was boiled for 8-10 minutes, cooled at RT and absorbance was taken at 625 nm on spectrophotometer (UV-1800 Shimadzu).Carbohydrate content was estimated by comparing with the standard glucose curve. Triplicates were maintained per gender/treatment/population.

3.7 *Protein Content*

Protein concentrations in the whole body homogenates were determined by the BCA method using bovine serum albumin as the standard. Briefly 10 flies were homogenized in O.1M PBS and then centrifuged for 12000 rpm at 4°C for 10 minutes. 150 μl of supernatant was used for biochemical assay, to which 3ml of Sigma BCA protein assay reagent was added and incubated for 40 minutes at RT. Protein concentration was determined by comparing absorbance at 562nm (UV-1800 Shimadzu) with standard curves. Triplicates were maintained per gender/treatment/population.

4. Results

4.1 *Developmental Time*

The pre adult development time differed significantly across population type ($F_{1,1= 52,6205.5}$ p=0.00). The JB populations took significantly longer time to complete development and emerge as adults compared to FLJ populations. There was significant treatment ($F_{2,2}$ = 70.6721 p=0.01) and treatment × gender ($F_{2,2}$ = 32,4328 p=0.02) effect (Figure 1). Over all the flies took significantly longer time to complete development in $FeSO_4$ supplemented media compared to SM and $CdCl_2$ supplemented media, and the two genders behaved differently in the different media types. There was no population type × treatment interaction effect.

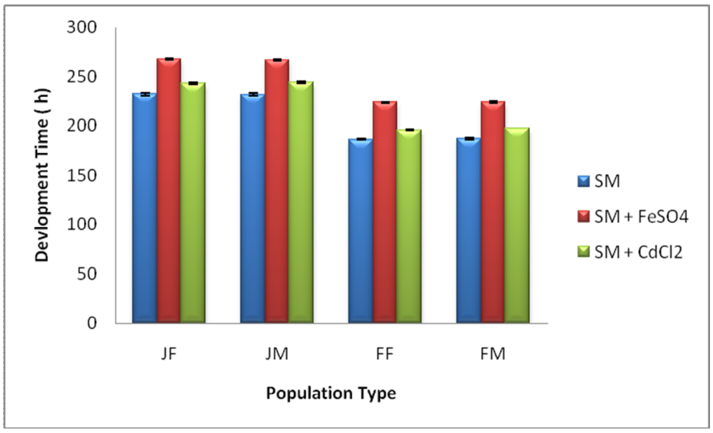

Figure1: Effect of heavy metals of developmental time on females and males in control and selected population respectively (Mean ± s.e., $p \leq 0.05$).

4.2 Dry weight

There was a significant main effect of population type ($F_{1,2}$= 626.4807 p=0.001) and fly gender ($F_{1,2}$ = 689.4358, p=0.001). Over all JB flies had significantly higher weights than their FLJ counter parts. Further, female flies had significantly higher lipid content than males. There was no significant effect of heavy metals on the dry weight of the flies.

4.3 Fluid content

There was a significant main effect of population type ($F_{1,2}$= 53.5021 p=0.018) and fly gender ($F_{1,2}$ = 1006.523, p=0.001). Over all JB flies had significantly higher fluid content than their FLJ counter parts. Further, female flies had significantly higher fluid content than males. There was no significant effect of heavy metals on the dry weight of the flies.

4.2 *Lipid Content*

The lipid biosynthesis seems to be unaffected by HM supplementation. There was a significant effect of population type ($F_{1,2}$= 608.7864 p=0.00) and fly gender ($F_{1,2}$ = 47.570, p=0.02). Over all JB flies had significantly higher levels of lipids than their FLJ counter parts. Further, female flies had significantly higher lipid content than males.

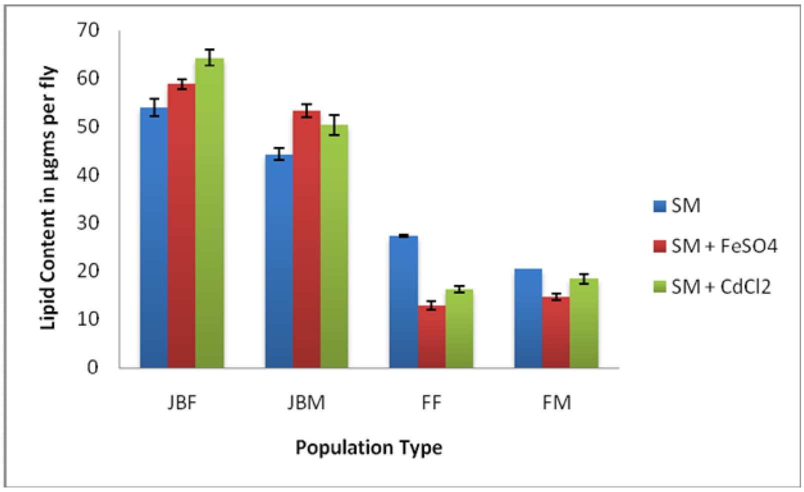

Figure2: Effect of heavy metals on Lipid content of females and males in control and selected population respectively (Mean ± s.e., $p \leq 0.05$).

4.3 *Protein Content*

The protein content was significantly increased with the application of HM stress. There was significant effect of population type ($F_{1,2}$ =9720.154 p=0.00). The JBs had significantly higher protein levels than their FLJ counter parts. Further, there was significant effect of treatment ($F_{2,4}$ =94.1043 p=0.00), gender ($F_{1,2}$ = 1317.93 p=0.00) with females having higher levels of protein than males, population type × treatment ($F_{2,4}$ =10.257 p=0.026) and population type × gender ($F_{1,2}$ =443.2162 p=0.00).

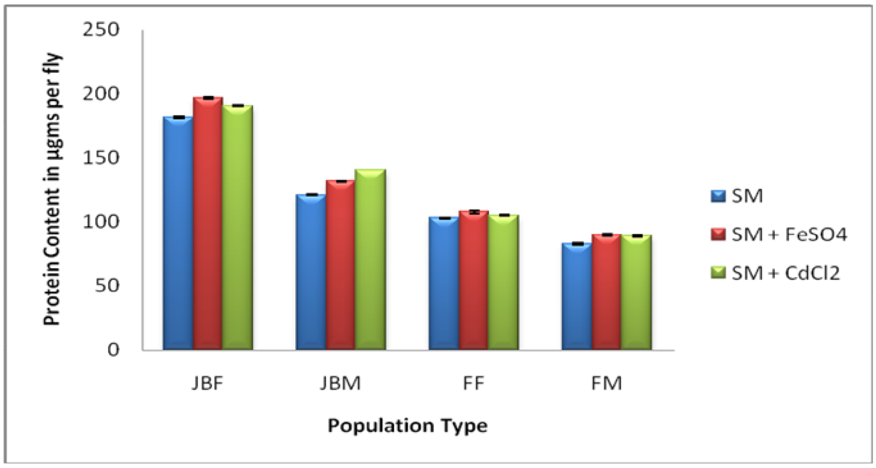

Figure3: Effect of heavy metals on Protein Content of females and males in control and selected population respectively (Mean ± s.e., $p \leq 0.05$).

4.4 *Carbohydrate Content*

The Carbohydrate content was seen to be decreased significantly with different treatments of HM across both JB and FLJ populations in both the genders. There was significant main effect of population type ($F_{1,2}$ =192.71 p=0.00), treatment ($F_{2,4}$ =1089.49 p=0.00), gender ($F_{1,2}$ = 933.06 p=0.00). Further, population type × treatment ($F_{2,4}$ =18.35 p=0.00), treatment ×gender ($F_{2,4}$ = 124.47 p=0.00) and population type × treatment× gender ($F_{2,4}$ =826.4 p=0.00) interaction effects were also significant.

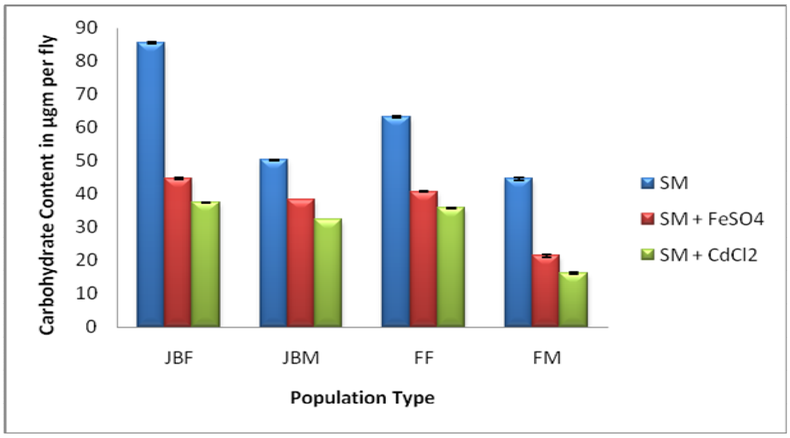

Figure 4: Effect of heavy metals on Carbohydrate Content of females and males in control and selected population respectively (Mean ± s.e., $p \leq 0.05$).

4.5 *Statistical Analysis*

Population mean values were used as unit of analysis and Univariate analysis model of ANOVA was carried out using SPSS 16.0.

5. Discussion

The present study showed that the carbohydrate content of the flies reared on heavy metal supplemented diet was significantly lower than those that were reared on SM, while the lipid and protein levels were marginally higher (in the JB populations). These results are in agreement with those of Marron *et al.,(*2006), suggesting that the bio-molecular synthesis of macro molecules and their utilization seems to be a well coordinated process across biological systems.

The elevated protein levels in the flies reared on heavy metal supplemented diet (Fig.3) might be due to increased level of Catalase, SOD and/or other proteins that are involved in maintain the homeostasis of the organism. The exposure of flies to various stress inducers have been show to hike up the ROS scavenging proteins in many systems (Kreg *et al.*, 2007). Our results clearly suggest that the stress tolerance mechanisms are perhaps conserved across animal systems.

Acknowledgements

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References

[1] Aisen P. , Enns C., Wessling-Resnick M (2001), Chemistry and Biology of eukaryotic iron metabolism. *The International Journal of Biochemistry & Cell Biology*, **33**, 940–959.

[2] Akins J M, Schroeder J A, Brower D L & Aposhian H (1992), Evaluation of Drosophila melanogaster as an alternative animal for studying neurotoxicity of heavy metals. *Biometals***, 5,** 111-120.

[3] Boggs, C L and Freeman, K D (2005), Larval food limitation in butterflies:effects on adult resource allocation and fitness. *Oecologia*, **144**, 353–361.

[4] Chandrashekara K T and Shakarad M. (2011), *Aloe vera* or Resveratrol Supplementation in Larval Diet Delays Adult Aging in the Fruit Fly, *Drosophila melanogaster Journal of Gerontology: Biological* Sciences,**66A (9),**965–971.

[5] Copat C., Bella F., Castaing M., Fallico R., Sciacca S., Ferrante M (2012), Heavy metals concentrations in fish from Sicily (Mediterranean Sea) and evaluation of possible health risks to consumers. *Bull. Environ. Contam. Toxol.* ***,88,*** 78–83.

[6] David J R, Allemand R.,Van Herrewege J. and Cohet Y (1983), Ecophysiology: a biotic factors. *In Genetics and Biology of Drosophila (ed. M. Ashburner, H. L. Carson and J. N. Thompson,* **pp.** 105-170. New York: Academic Press.

[7] Djawdan M., Chippindale A K, Rose M R, and Bradley T J (1998), Metabolic reserves and evolved stress resistance in Drosophila melanogaster. *Physiol. Zool.*, **71,** 584-594.

[8] Farombi E O, Adelowo O A, Ajimoko Y R (2007), Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat Fish (*Clarias gariepinus*) from Nigeria Ogun River. *Int. J. Environ. Res. Public Health***, 4,** 158–165.

[9] Harshman L G, and Schmid J L (1998), Evolution of starvation resistance in Drosophila melanogaster: Aspects of metabolism and counter-impact selection. *Evolution* , **52,** 1679-1685.

[10] Hendry G A F, Baker A J M, Ewart C F (1992), Cadmium tolerance and toxicity, oxygen radical processes and molecular damage in cadmium- tolerant and cadmium-sensitive clones of *Holcus anatas. Acta Bot. Neerl.*, **41,** 271-281.

[11] Hoffman A A & Parsons P A (1991), Evolutionary Genetics and Environmental Stress. *Oxford University Press, Oxford, UK.*

[12] Ives P T (1970), Further studies of the South Amherst population of Drosophila melanogaster. Evolution, **38,** 507-518.

[13] Kregel K C and Zhang H J (2006), An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations *Am J Physiol Regul Integr Comp* Physiol , **292,** R18–R36.

[14] Koehn R K and Bayne R L (1989),Towards a physiological and genetical

understanding of the energetics of the stress response. *Biol. J. Linn. Soc.,* **37,** 157–171.

[15] Marron M T, Markow T A, Kain K J,Gibbs A G (2003), Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila. Journal of Insect Physiolog* , **49,** 261-270.

[16] McGraw L A, Fiumera A C, Ramakrishnan M, Madhavarapu S, Clark A G, and Wolfner M F(2007), Larval rearing environment affects several post-copulatory traits in Drosophila melanogaster. *Biology Letters*, **3,** 607-610.

[17] Opfer S E, Farver J R, Jeffrey G, Krieger M K (2011), Heavy metals in sediments and uptake by burrowing mayflies in western Lake Erie basin. *J. Great Lakes Res*, **37,** 1–8.

[18] Papanikolaou N C, Hatzidaki E G, Belivanis S, Tzanakakis G N, and Tsatsakis A M (2005), Lead toxicity update. A brief review. *Med. Sci. Monit.*, **11,** RA329-RA336.

[19] Parsons P A (1983), The Evolutionary Biology of Colonizing Species. *Cambridge: Cambridge Univ. Press*

[20] Patra J, Maheswar L, Panda B B (1994), Tolerance and co-tolerance of the grass *Chloris barbata* Sw. to mercury, cadmium and zinc. *New* Phytol, **128,** 165–171.

[21] Prasad N G, Shakarad M., Anitha D., Rajamani M. and Joshi A (2001), Correlated responses to selection for faster development and early reproduction in *Drosophila*: the evolution of larval traits. *Evolution,* **55,** 1363-1372.

[22] Rajamani M.,Raghavendra N., Prasad N G, Archana N., Joshi A., Shakarad M (2006), Reduced larval feeding rate is a strong evolutionary correlate of rapid development in Drosophila melanogaster *Journal of Genetics,* **Vol No. 85,** No. 3.

[23] Rion S, and Kawecki T J (2007), Evolutionary biology of starvation resistance: what we have learned from Drosophila. *J. Evol. Biol.,* **20,** 1655-1664.

[24] Satarug S, Baker J R, Reilly P E B, Esumi H & Moore M R (2000), Evidence for a synergistic interaction between cadmium andendotoxin toxicity and for nitric oxide and cadmium displacement of metals in the kidney. *Nitric Oxide: Biology and* Chemistry, **4,** 431-440.

[25] Sibly R M and Calow P (1989), A life cycle theory of responses to stress. *Biol. J. Linn. Soc.,* **37,** 101–116.

[26] Sokolowski M B, Kent C, Wong J (1984), *Drosophila* larval foraging behavior—developmental stages. *AnimBehav* , **32,** 645–51

[27] Van Remmen H, Hamilton M L & Richardson A (2003), Oxidative damage to DNA and aging. *Exerc Sport Sci Rev.,* **31,** 149-153.

[28] Zera A J and Harshman L G (2001),The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics* , **32,** 95–126.

[29] Zwaan B J, Bijlsma R, Hoekstra R F (1995), Artificial selection for developmental time in D. melanogaster in relation to the evolution of ageing: direct and correlated responses. *Evolution* , **49,** 635–648.