Biol Trace Elem Res (2013) 154:81–87
DOI 10.1007/s12011-013-9700-1

Elemental Concentration in Mealworm Beetle (*Tenebrio molitor* L.) During Metamorphosis

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Received: 4 April 2013 / Accepted: 7 May 2013 / Published online: 23 May 2013

Abstract Mealworm beetles have been used in numerous experiments as bioindicators. The aim of our experiment was to study the elemental composition in three larvae, pupae and first and second generation adult stages during their life cycle. We selected 180 larvae from a genetically similar population and put them in three groups, in two boxes (60 larvae in each box). Larvae were fed with mashed potato made of the same quality and quantity of potato powder. Then, we selected 10 individuals from each stage to the elemental analysis, using the ICP-OES method. The following elements were analysed in the studied stages: Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr and Zn. The results of principal component analysis demonstrated that based on elemental composition, different stages were separated with each other, but in the cases of the three larvae stages, high overlap was found. The results of the GLM ANOVA showed significant differences between the different stages of metamorphosis-based elemental composition. Our results show that the calcium and magnesium were found in a relatively high concentration, while the iron and zinc may be essential elements during the metamorphosis. Our results also show that in insect, the concentration of sodium was higher than in the pupa which may cause by hemolymph. We also demonstrated that the metamorphosis has an effect on the concentration of elements. Our study shows that in the different stages of insects, there are significant changes in the elemental composition of different stages of insects during their metamorphosis.

Keywords Larvae · Pupae · Adult · ICP-OES

Introduction

Terrestrial invertebrates are widely used as bioindicator organisms in ecological and pollutants studies. They are good indicators because of their high diversity and they have an important functional role in the terrestrial ecosystem [1]. Among terrestrial invertebrates, insects play an important role in elemental transport chains [2]. Insect populations are sensitive to their habitat quality and they can indicate the changing of their habitats with their quantitative and qualitative parameters. The studies of insects as bioindicators can provide information about ecosystem status [3].

Mealworm beetles (*Tenebrio molitor*) are good model organism because of its short life cycle and easy collection [4]. However, the larvae of *T. molitor* are commercially available and its reproduction is easy [5]. In many laboratory studies, mealworm beetle was used as the model organism [5–8]. Sheiman et al. [9] studied the host–parasite interaction with *T. molitor* and *Hymenolepis diminuta*. In another study, the effect of electromagnetic radiation was study on learning and memory ability of *T. molitor* larvae and adults [10]. In contrast with other beetles, the moult of number of *T. molitor* larvae may change depending on the quality of food. So, a kind of defense strategy was observed in the case of this beetle [11].

Castilla et al. [5] studied the effects of elements, nitrate and herbicides on growth in *T. molitor* larvae. This study demonstrated that the K and Zn may impact on the growth and survival of *T. molitor* larvae. An earlier study also demonstrated that the K and Zn had effect on the growth of larvae [6]. However, the metamorphosis plays an important role in the metal accumulation in holometabolous insects, which is particularly important in environmental studies [2].

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The effect of metamorphosis on concentration of elements and other compounds has been little studied. So, the aim of our study was to analyse the elemental concentration in mealworm beetle (*T. molitor*) during its development stages. Among developmental stages, the elemental concentration of three larvae, pupae and first and second generation adult individuals was analysed. Our hypotheses are the following: (a) In all stages, the elemental concentration depends on the needs of the stages. (b) Conversion rate of main elements is high from foods, and difference may be high during metamorphosis.

Materials and Methods

Life Cycle of Mealworm Beetle

Flour beetles develop with complete metamorphosis which involves four life cycle stages: egg, larvae, pupa and adult. Female beetles lay 300–400 eggs, from which larvae individuals are developed within 5 to 12 days. The larval period varies from 22 to 100 days, while the pupa period is about 8 days. Among larvae, three stadiums were applied in the experiments [12]. The first, second and third larvae stages were separated by morphological parameters. The determination of instars is possible with sensitive and specific mass fragmentographic method [13]. So, in the case of larvae stages, the results of earlier studies were used to identify instars [14, 15]. In our study, the third instar (6.8±1.9 mm, mean ± SD), the eighth instar (7.8±1.3 mm, mean ± SD) and the last instar (20.4±1.2 mm, mean ± SD) were used. In the manuscript, the third instar was called first larvae stadium, the eighth instar was the second larvae stadium and the last instar was third larvae stadium. For the pupae determination, Wigglesworth's [16] observation was used. We studied the last pupae stage where the head is darkened and the pupal cuticle was soft and wrinkled [13].

Experiments

Our experiments are based on a genetically similar mealworm beetle's culture. There were 60 randomly selected specimens from the first stadium of larvae and placed into open plastic boxes. During the experiment, two boxes were replicated three times to elemental analysis. There were 60 larvae individuals in each box at the start of the experiments. All boxes were kept under similar environmental conditions. The mean temperature was 27 °C and the humidity was 80 % by spraying with distilled water every 48 h. Larvae and adult beetles were fed with similar type and quantity of food twice a week. It is known that the cannibalism rate is usually high (25–35 %) in mealworm beetle population [17]. To minimize cannibalism, the pupas were moved into a small plastic box. In all boxes, feeding was run simultaneously and managed in the same way. Food was prepared from mixed potato powder. For each treatment, 5.0 g mixed potato powder was weighted in the small plastic box and mixed with deionised water. The used food was dry, but this was a mixed powder. It was a commercial product and it was also used as food for human. Similar to our study, potato was also used as food in other studies [18, 19]. We preferred to use this type of food because with the mashed potatoes, we could also provide the need of liquid for larvae and beetles.

Trace Element Analysis

From the three larvae stadiums, pupa and beetles, 10 individuals were selected randomly for the analysis.

From each stage, individuals were collected before feeding, so the starving was ensured. Probably, the gut did not contain food. Strong enzymatic activities are specific in the first instar insect while this activity disappears in the pupae stage during metamorphosis [20]. Some studies also reported enzymatic activities during metamorphosis from larvae stage to pupae, but the pupal enzyme activities did not differ during pupation [21, 22].

Until processing, samples were stored in a freezer at −15 °C. Each sample was placed in a plastic sieve and flushed with 100 mL of double deionised water obtained from a Millipore Milli-Q system. In the case of first, second and third larvae stadium and integument of first, second, third larvae and pupa stadium, average samples were analysed because of the small weight of samples. All samples were transferred individually into a 25-mL beaker. The wet body mass of the pupa and beetles was measured immediately. All samples were dried overnight at 105 °C and the pupas and beetles were reweighted to determine their dry mass. The dry weights of larvae and integuments were measured with a SARTORIUS LE 26P micro-analytical balance. Then, all samples were digested using 2 mL of 65 % (*m*/*m*) nitric acid (Scharlau). In the case of second and third stadium larvae, 0.5 mL of 30 % (*m*/*m*) hydrogen peroxide was also used. The digestion was in the same container at 80 °C for 4 h. Digested samples were diluted to 5 mL (first and second stadium of larvae and integuments) and 10 mL (third larvae stadium, pupa and beetles) using a 1 % (*m*/*m*) nitric acid. For the analysis of contaminated food, similar sample processing was used [23, 24].

Analysis of the elements was performed by ICP-OES IRIS Intrepid II XSP equipped with a CETAC 45 000 AT + ultrasonic nebulizer. We used a seven-point calibration procedure (0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 mg L^{-1}) with a multi-element calibration solution (Merck ICP multi-element standard solution IV). The analysis was performed using two or three atomic or ionic lines of the corresponding elements. In the case of alkaline metals (e.g. Li, Na and K), we used single lines. The selected lines were free of spectral interferences in these sample matrices. The limits of quantification values are given in milligrams per liter: Ba, 0.006; Ca, 0.008; Cd, 0.001; Co, 0.004; Cr, 0.002; Cu, 0.003; K, 0.003; Li, 0.002; Mg, 0.001; Mn, 0.001; Na, 0.002; Ni, 0.004; Pb, 0.013; Sr, 0.002; and Zn, 0.003 [23, 24].

Statistical Analysis

SPSS/PC statistical software package was used during the calculations. Principal component analysis (PCA) was used for evaluating the elemental concentration of beetles in the different stages. Thus, concentration of data was normalized for the elemental concentrations of the stages of mealworm beetles. Homogeneity of variances was tested by Levene test. The elemental concentrations of beetles in the stages were compared with ANOVA. In case of significant differences, Tukey's multiple comparison test was used. Pearson rank correlation was used to study the correlation between elemental concentration of food and studied stages of beetles [25].

Results

Principal Component Analysis

The first component (PC1) contributed 85.4 % of cumulative variance and the second one (PC2) contributed 8.3 %. The eigenvalue was 0.854 in the case of PC1, while the result of eigenvalue was 0.084 in the case of PC2. The results show the total separation of stages from each other based on elemental concentrations of samples (Fig. 1).

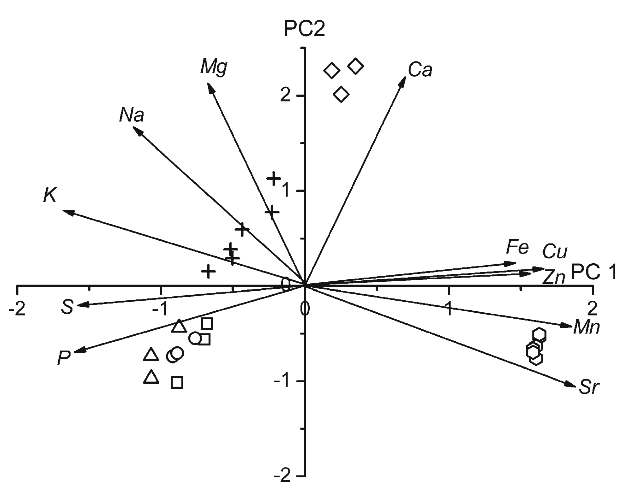


Fig. 1 Principal component biplot of the stages of mealworm beetle and score of elemental concentrations of samples. *White square*, first stage of larvae; *white circle*, second stage of larvae; *white up-pointing triangle*, third stage of larvae; *white diamond*, pupae; *hexagon*, first generation adults; *plus sign*, second generation adults

Although high overlap was found among the stages of larvae, the PCA shows that Fe, Cu, Zn, Mn and Sr correlated with the PC1. This correlation indicates that the concentrations of these elements were significantly higher in the first generation adults than other stages. Negative correlation was between the concentration of P, S and K and PC1. Thus, these element concentrations were lower in the first generation adults than in other stages. In the case of Ca, positive correlation was found between concentration and PC2, which indicates that the Ca concentration was the highest in the stage of pupae. The Na and Mg concentration negatively correlated with PC2, so these element concentrations were significantly lower in the stage of pupae than in other stages.

Elemental Concentration in the Stages of Mealworm Beetles

Significant differences were found in all concentration of element with ANOVA ($p<0.001$) (Table 1). Using Tukey's multiple comparison test, the Fe, Mn, Sr and Cu concentration was decreased significantly from first larvae stage to third larvae stage. In the cases of all elements (Ca, K, Mg, Na, P and Zn), significant differences were not found among larvae stages ($p>0.05$). The K, Na, S and Sr concentration significantly decreased from larvae stages from the

Table 1 ANOVA of elemental concentrations in different stages of mealworm beetles (in milligrams per kilogram, dry weight)

Elements			*df*	*F*	*p* value
Ca	Stages	Hypothesis	5	1,005.492	<0.001
		Error	5		
Cu	Stages	Hypothesis	5	333.0181	<0.001
		Error	5		
Fe	Stages	Hypothesis	5	304.3315	<0.001
		Error	5		
K	Stages	Hypothesis	5	3,320.622	<0.001
		Error	5		
Mg	Stages	Hypothesis	5	550.5617	<0.001
		Error	5		
Mn	Stages	Hypothesis	5	168.1551	<0.001
		Error	5		
Na	Stages	Hypothesis	5	4,727.923	<0.001
		Error	5		
P	Stages	Hypothesis	5	1,423.905	<0.001
		Error	5		
S	Stages	Hypothesis	5	2,335.179	<0.001
		Error	5		
Sr	Stages	Hypothesis	5	587.5947	<0.001
		Error	5		
Zn	Stages	Hypothesis	5	1,216.785	<0.001
		Error	5		

development of pupae ($p<0.05$). However, in the first and second larvae stages, the Ca and Mn concentration was significantly higher than the pupae ($p<0.05$). Significant difference was not found in the Cu concentration between first larvae and pupae, but the Fe and Zn concentration was higher in the first larvae stage than in the pupae. In the cases of all elements, significant differences were found among larvae stages and first generation adult beetles ($p>0.05$) (Table 2).

Concentration differences were found between pupae and first and second generation beetles in the cases of all elements, except Fe and Zn ($p>0.001$). The Fe concentration was significantly higher in the first generation adults than in the pupae and in the second generation adults ($p<0.001$). In contrast with this, the Zn concentration was higher in the pupae and second generation adults than in the first generation adults ($p<0.001$) (Table 2).

Compared to the element concentration of first and second generation adults, the Ca, Fe and Mg concentration significantly decreased in the second generation adults ($p<0.001$), while the concentrations of other elements were significantly higher in the second generation adults than in the first generation adults ($p<0.001$) (Table 2).

Correlation Between Elemental Concentration of Food and Beetles

To estimate the food conversion rate, we studied the correlation between elemental concentration of food and different stages of beetles. In all stages, positive correlation was found between elemental concentration of food and studied stages of beetles (Figs. 2 and 3). In the first larvae stage, the correlation was strong (Pearson: $p<0.001$, $r=0.955$), and Fig. 2a shows that in this stage, the conversion rate of Mn, Zn, Fe and Ca is almost 100 % from food. In the second, the correlation was also significantly positive (Pearson: $p<0.001$, $r=0.936$), but the conversion rate of Mn, Ca and Mg was also 100 % from food, but in the cases of other elements, such as Zn and Fe, this rate was lower or higher than in first larvae stage (Fig. 2b). The conversion rate of this element decreased in the third stage because only in the case of Ca and Mg that conversion rate was 100 %, but the correlation was positive (Pearson: $p<0.001$, $r=0.918$) (Fig. 2c). The conversion rate of Sr and Na was lower than 100 %, while the conversion rate of Cu, S and P was higher than 100 % in all larvae stage.

Similar to larvae stages, the correlation between elemental concentration of food and stages was also positive in the pupae (Pearson: $p<0.001$, $r=0.927$) and first (0.945) and second (0.936) generation adults (Fig. 3). The conversion of Fe, Zn and Ca was 100 % from food in the pupae and adult stages, but the conversion of Sr, Mn and Na was lower than 100 %. However, the conversion of Mg only in the second generation adult was lower than 100 %. Conversion rate of other elements (Sr, Na, Cu, S and P) was similar in the pupae and adult beetles than larvae stages.

Discussion

In our study, the change of elemental concentration was analysed in mealworm beetles during their metamorphosis. We studied the following element concentrations, which have important role during the metamorphosis: Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr and Zn. The inorganic compounds such Ca, Mg, Na, chlorides and phosphates are essential for function of cell, so they are important parts of foods for insects [26].

Among main elements, the hemolymph of insect contains high concentration of Ca and Mg [27]. In the case of Na, an earlier study demonstrated that the concentration of this element is low, especially in phytophagous insects.

Table 2 Concentrations of elements (mean ± SD) in the stages of mealworm beetles

Elements	First larvae	Second larvae	Third larvae	Pupae	First generation Adult	Second generation Adult
Ca, mg kg^{-1}	736±193	583±52	449±88	348±72	799±129	524±198
Cu, mg kg^{-1}	14.4±4.1	10.9±1.8	10.6±2.8	15.2±9.8	19.5±11.7	37.3±15.4
Fe, mg kg^{-1}	250±209	98±29	46±10	108±96	115+95	106±46
K, g kg^{-1}	14.6±2.2	14.5±1.2	12.5±2.6	3.5±0.2	9.0±1.6	9.9±2.9
Mg, g kg^{-1}	1.9±0.3	1.8±0.2	2.0±0.6	2.2±1.0	1.7±0.7	1.6±0.8
Mn, mg kg^{-1}	18.9±5.9	14.7±3.1	10.3±1.9	9.9±2.9	11.9±6.0	12.7±2.4
Na, g kg^{-1}	2.1±0.4	1.6±0.2	1.6±0.4	0.8±0.2	1.2±0.4	2.7±0.7
P, g kg^{-1}	65±28	77±35	62±31	1.8±0.2	6.0±1.0	18.0±9.9
S, g kg^{-1}	9.1±6.4	6.3±1.6	6.6±3.0	1.0±0.7	1.9±0.2	4.7±1.4
Sr, mg kg^{-1}	8.8±2.4	7.1±6.7	5.7±4.9	1.4±0.6	1.4±0.5	4.1±1.4
Zn, mg kg^{-1}	171±57	115±10	94±26	92±12	81±9	90±22

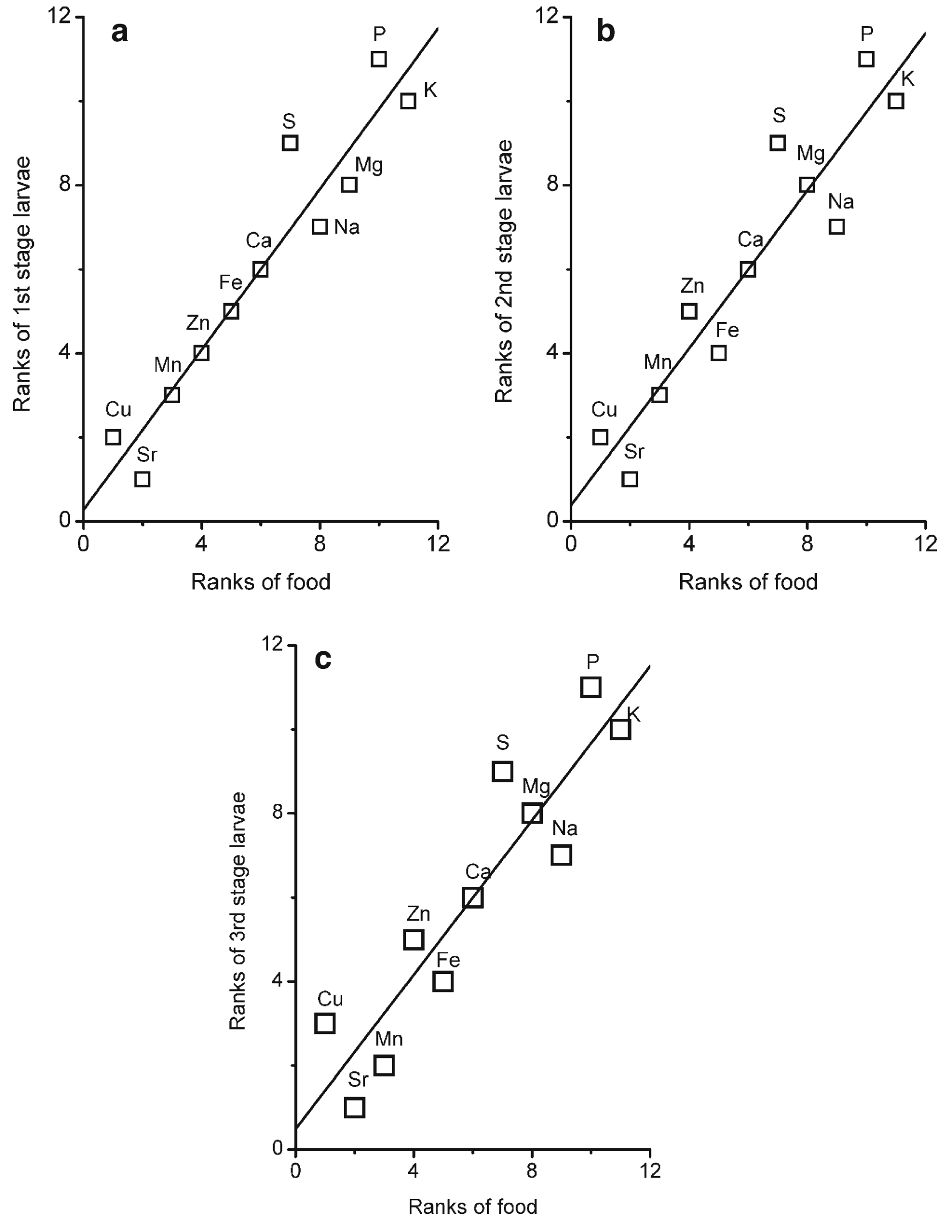
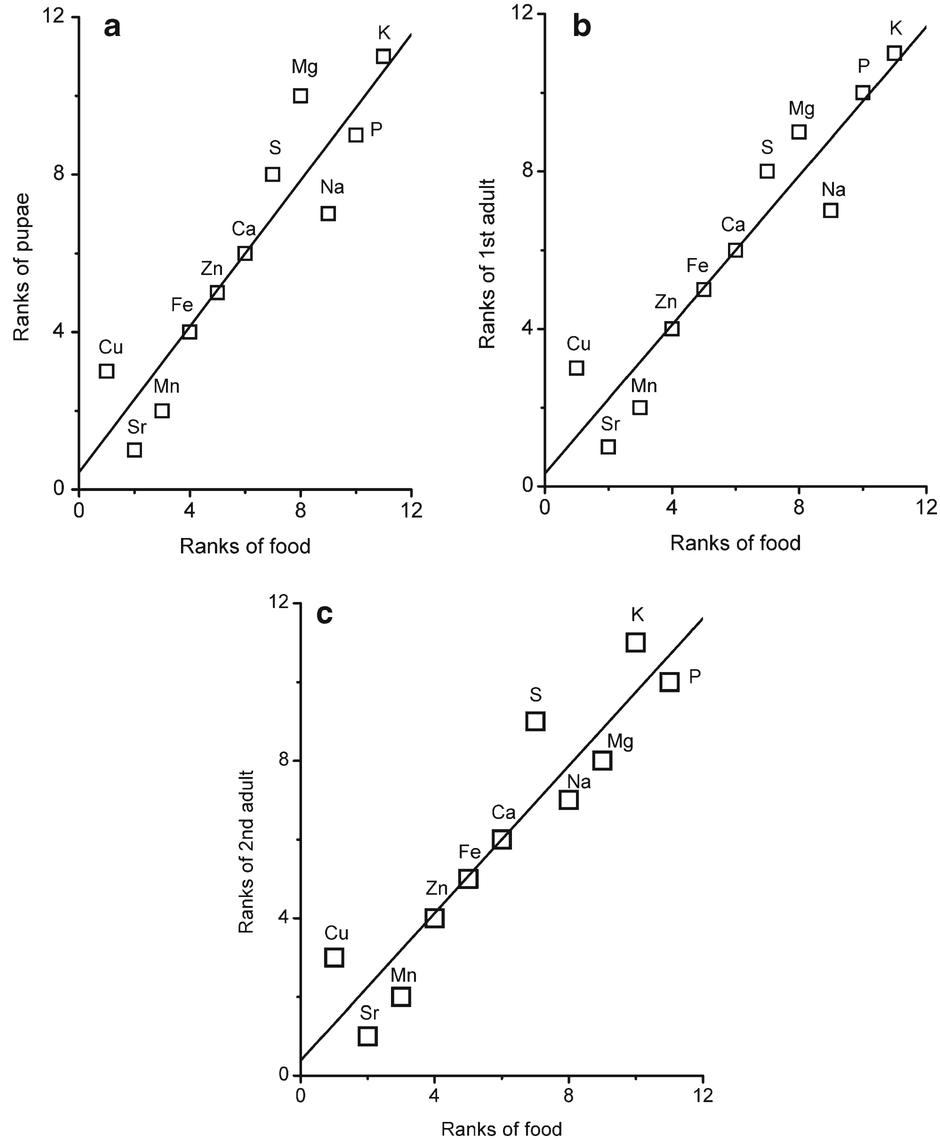


Fig. 2 Rank correlation between elemental concentration of food and studied larvae stages. **a** First larvae stage. **b** Second larvae stage. **c** Third larvae stage

However, the Na concentration is different in complete metamorphosis insects [27]. In the hemolymph of insect, the Na concentration is significantly higher than in the larvae. In contrast with it, the Mg concentration is significantly higher in the larvae than in the adult beetles in the Coleoptera order [27]. We found that the concentration of K was decreased to due pupae development, while before moult, the concentration of this element may decrease in some species. The change of K concentration may influence the behaviour of insect because of the interaction between neuromuscular points and low K concentration, so the resting potential may increase during moult [27]. The Zn and Fe are essential for insects especially in the insect hemolymph. However, the different parts of cuticle may contain high concentration of Zn, Fe and Mn [27].

In our study, higher Zn and Cu concentration was found in mealworm beetle larvae than in the findings of other studies, where mosquito and insect larvae were studied [28, 29]. Nummelin et al. [30] found lower Mn concentration in dragonfly larvae, but the Fe concentration was higher than in our findings. This difference may caused by an iron company which was near their sampling site [30]. Lindqvist and Block [2] demonstrated the Zn concentration was not affected by metamorphosis. In contrast with their findings, our study indicated that the Zn concentration was higher in the first larvae stage than in pupae. However, our results also demonstrated that in the pupae and second generation adult, Zn concentration was higher than in the second generation adult, so Zn concentration may have a role in the metamorphosis. Similar to our study, Lindqvist and Block [2] also demonstrated that during

Fig. 3 Rank correlation between elemental concentration of food and pupae, first and second generation adults. **a** Pupae stage. **b** First generation adult. **c** Second generation adult



metamorphosis, remarkable difference may be in elemental concentration among developmental stages. A study of Polydesmida species also found differences in Ca, Mg, K, Na, Zn and Cu concentration between larvae and adult [2].

There is a little information about food conversion ratio in insect studies. Ernst [31] demonstrated that weevil larvae (*Stegobium paniceum*) could utilize more than 90 % of Cu, 50 % of Fe and 20 % of Ca, Mg and K from foods. Using correlation between elemental concentration of food and beetles, our results demonstrated higher food conversion ratio in the case of Cu, Fe Mn and Ca in contrast with an earlier study [31]. But similar to this study in the case of K, we also found low conversion ratio in all studied stages.

Our result showed that the elemental concentrations of mealworm beetle were remarkable during their metamorphosis. However, the study of correlation between elemental concentrations of food and beetles also demonstrated that some elements (Ca, Cu and Mn) utilized about 100 % from foods, but this conversion ration depends on the stages. In summary, a remarkable difference is in the elemental concentration of beetle among developmental stages during metamorphosis.

Acknowledgments The work is supported by the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 and TÁMOP-4.2.2.A-11/1/KONV project. These projects are implemented through the New Hungary Development Plan, co-financed by the European Social Fund and the European Regional Development Fund.

Conflict of interest The authors declare no conflicts of interest.

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