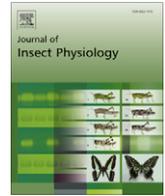


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## Energy storage and C:N:P variation in a holometabolous insect (*Curculio davidi* Fairmaire) larva across a climate gradient

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## ABSTRACT

Increasing empirical evidence has documented variability in elemental composition within species. However, the extent, causes, and pattern of variability in consumer stoichiometry across a large geographical scale are not well understood. Here, we investigated this issue using a holometabolous insect, weevils (*Curculio davidi* Fairmaire). Larvae of this species store energy needed for diapause, and variable energy requirements across the geographic range of this species could lead to differences in body elemental composition. Our results showed that variability was high (assessed as the coefficient of variation (CV)) in larval body nitrogen (N) and phosphorus (P) (CV, 10% for N and 13% for P) compared to emerging adults (CV, 5% for N and 8% for P). Temperature-related factors explained more variation than other climatic factors and food for carbon (C), N and P in weevil. In warmer regions, larval C concentration was higher, while N and P were lower. The high C content of weevil larvae relative to both their food source and their adult stage was attributed to energy storage. Across the climatic gradient of its geographic range, larval body C content increased with mean annual temperature and decreased with average diurnal temperature range. This finding implies that temperature-related C storage drives the high variability in elemental composition of larvae across the climate gradient, and also effectively dampens the stoichiometric imbalance between consumers and food resources while serving as an energy reservoir for overwintering and metamorphosis.

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## 1. Introduction

Many studies have accessed intraspecific variation in stoichiometric composition within a habitat or across different habitats (Hambäck et al., 2009; El-Sabaawi et al., 2012). This variation can be attributed to differences in developmental stage (Villar-Argaiz et al., 2002; Laspoumaderes et al., 2010; Nakazawa, 2011), food quality (Schade et al., 2003; Persson et al., 2010), environmental conditions (e.g., temperature) (Chrzanowski and Grover, 2008; Persson et al., 2011), or demands for storage in the face of variability in food supply accompanying seasonal effects (Sterner and Schwalbach, 2001; Ventura and Catalan, 2005; Hood and Sterner, 2010). However, the extent, causes, and pattern of variability in consumer stoichiometry across a large geographical scale are poorly understood (Supplementary data).

Temperature is a principal environmental variable driving regional physiological adaptations by controlling metabolic rate,

thereby modifying the elemental demand of organism (Gillooly et al., 2001; Mcfeeters and Frost, 2011). Many studies showed that plant nitrogen (N) and phosphorus (P) concentrations decline with increasing temperature, but carbon (C) concentration is almost unchanged (Han et al., 2005, 2011; Yu et al., 2010; Sun et al., 2012). The climate-related variation in plant stoichiometry may also affect the consumer stoichiometry (Loladze, 2002; Tao and Hunter, 2012). Furthermore, stored energy in the form of C-rich lipids in animals that undergo diapause may alter the relationship between body elemental composition and temperature, when energy storage responds to metabolic demands of diapause (e.g., Zhou et al., 1995; Denlinger, 2001; Hahn and Denlinger, 2007, 2011). Therefore, we expect that temperature variability may change the stoichiometric composition of the diapause animal either via effects on food composition, or directly on the metabolic and storage demands of the animal.

The acorn weevil (*Curculio davidi* Fairmaire) is a holometabolous phytophagous insect that is widely distributed throughout eastern Asia. Development appears to occur in a 2-year life cycle. In middle summer, females usually oviposit in acorns, and weevil larvae hatch from the eggs a few days after they are laid. The larva is

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endoparasitic, which completes the development inside a single host acorn (progress through 5 instars) (Cheng and Hsu, 1956). The last developmental stage of weevil larvae can store much fat for overwintering as well as later development and reproduction. The larvae leave the fruit after the acorn fall and bury into the soil. After an overwintering larval diapause, most larvae undergo full metamorphosis during the late summer (Menu and Debouzie, 1995).

In mainland China, acorn weevils and oriental oaks stretch from temperate regions in the north to subtropical areas in the south (24° N to 42° N and 96° to 140° E) (Wang et al., 2009), and showed synchronized relationship. We hypothesized that across a temperature gradient, weevil larvae stoichiometry will display more variation, and also a tighter relationship with climatic factors, relative to emerging adults. Specifically, we hypothesized that weevil larvae C and lipid concentrations were higher in warmer regions. Therefore, our study aimed to (1) examine the variation in weevil (last-stage larvae and emerging adults) stoichiometry and larval lipid storage across temperate-subtropical biomass, (2) compare the differences of elemental composition and lipid storage in larvae and adults. (3) The organism usually stores substances with different stoichiometric traits of biochemicals (lipids, chitin, etc.) in particular structures (Faerovig and Hessen, 2003). Therefore, we also expect the higher C in the body than in the head, because the lipid is stored in the fat body. Measuring elemental composition in a widespread holometabolous species, which lives across a climatic gradient, can help us understand the patterns in body stoichiometry resulting from these climate-dependent biological demands.

## 2. Materials and methods

### 2.1. Study sites

Twenty one sites were selected in East China, stretching from temperate to subtropical areas (Fig. 1). Across this geographical zone, mean annual temperature (hereafter MAT, °C) ranges from 9.64 to 16.32 °C. In the temperate areas, the vegetation was deciduous broadleaf forests dominated by oriental oak and the typical soil was cinnamonic soil. In the transitional areas (from warm temperate to subtropical zone), the dominant vegetation was mixed deciduous and evergreen broadleaf forests, and brown soil was the dominant soil type. In the subtropical areas, the vegetation was evergreen broadleaf forests with oriental oak as a companion species, with red soil being the dominant soil type. All the sampled stands were relatively undisturbed secondary forests with no direct harvesting, burning, or other such activities for the last three decades

### 2.2. Sample collection and analysis

In each site, three plots (20 × 20 m) were chosen in the middle of a south-facing slope. We collected acorns that had naturally dropped to the ground, during the period from late September in north to late October in the south, in 2009. Approximately 300 acorns were haphazardly collected from each plot then pooled to prepare a bulk sample. About 200 healthy acorns were selected from each for chemical analysis. The remaining acorns were stored at room temperature several days to let most larvae chewed their way out of these acorns. By the time that collected acorns, larvae have nearly completed development. Therefore, the relatively short amount of time that acorns are held in the laboratory before the larvae emerge is unlikely to mask the influence of the natal climate on weevil stoichiometry. In all cases, specimens with similar body weights were starved overnight prior to chemical analysis to minimize the effects of the gut contents and covariation with body

size (Fagan et al., 2002). Larvae were collected after emerging from acorns and divided into three groups. From one group, 50 individuals were selected for chemical analyses. One group was stored in the –70 °C freezer was used for lipid analyses with three repetitive (5 individuals for one repetitive). The other group was placed in a 50 cm × 50 cm rubber container with air holes drilled on one side and filled with soil, and watered regularly to keep the soil moist. These larvae burrowed into the soil to pupate in the laboratory temperature after about 90 days, and then the adults were collected. We acknowledge that this “common garden” approach is likely to affect the stoichiometry of adult weevils, but collecting adult weevils directly from forests is not feasible, because we cannot control the developmental stage at so large geographical scale. Our experiment therefore accesses whether the variation in emerging adults is lower and less relationships with climatic factors after much storage energy was fueled than larval variation. For most of the sites, the adult samples were not enough to analyze the elements and lipid simultaneously, therefore, three sites (Pinggu, Beijing; Anning, Yunnan; Huoshan, Anhui) were chosen to investigate the lipid content of emerging adults.

All the specimens used for chemical analyses were cleaned with distilled water and dried at 50 °C for 72 h. They were weighed to the nearest 0.1 mg, and then returned to the drying oven for an additional 24 h. Reweighing was conducted to confirm that the specimens had reached a constant dry mass (Boswell et al., 2008). Because the head is very hard to grind for elemental analysis, each larva was separated into head and body, which can easily be distinguished based on differences in color. The head and body were pooled to prepare a bulk sample, respectively. Six small stir beads were each placed with each sample inside a vial. The vial was capped, then vortexed for 1 min to completely homogenize the dry sample, stored in a silica gel bag, and kept cool (4 °C) until chemical analysis.

Concentrations of C and N (mg g<sup>-1</sup>) were determined using an elemental analyzer (Vario EL III, Elementar, Germany). An approximately 5 mg subsample was weighed to the nearest 0.001 mg, wrapped in a small sheet of tin foil, and placed individually into automatic samplers. The samples were oxidized and reduced to N<sub>2</sub>, CO<sub>2</sub>, and SO<sub>2</sub>, respectively, and were measured using thermal conductivity and infrared detectors. Phosphorus concentration (mg g<sup>-1</sup>) was quantified using an inductively coupled plasma atomic emission spectrometer (Iris Advantage 1000, USA). The standard BCR-60, *Lagarosiphon major*, from the Community Bureau of Reference (BCR, Belgium) was used as a reference for spot-checking. The samples were digested using trace-metal-grade nitric acid and diluted in 100 ml of distilled water. Macro-element concentrations were expressed in milligram per gram dry weight. The mass of reference materials (0.1 g) was obtained to the nearest 0.1 mg. For larvae, whole animal C, N and P concentrations were calculated by the following formula:

$$y = \frac{(w_1 \times x_1) + (w_2 \times x_2)}{(w_1 + w_2)}$$

where  $y$  is the element concentrations in the whole organism,  $w_1$  and  $w_2$  were masses of head and body, respectively,  $x_1$  and  $x_2$  were the elemental concentrations in head, and body, respectively.

Lipid content in larvae was analyzed using the colorimetric techniques developed for fly analysis as modified by Nestel et al. (2003). The fresh samples were cleaned and weighed, then homogenized with 12 small magnetic stir bars and 2% Na<sub>2</sub>SO<sub>4</sub> in vial. Lipid was extracted with a chloroform–methanol separation method, and quantified using vanillin in phosphoric acid. The glucose and glycogen were extracted with distilled water, and measured using Anthrone in sulfuric acid method (Nestel et al., 2003). We also chose 45 oven-dry larvae from one site (Jinzhai, 31°9'28"N;

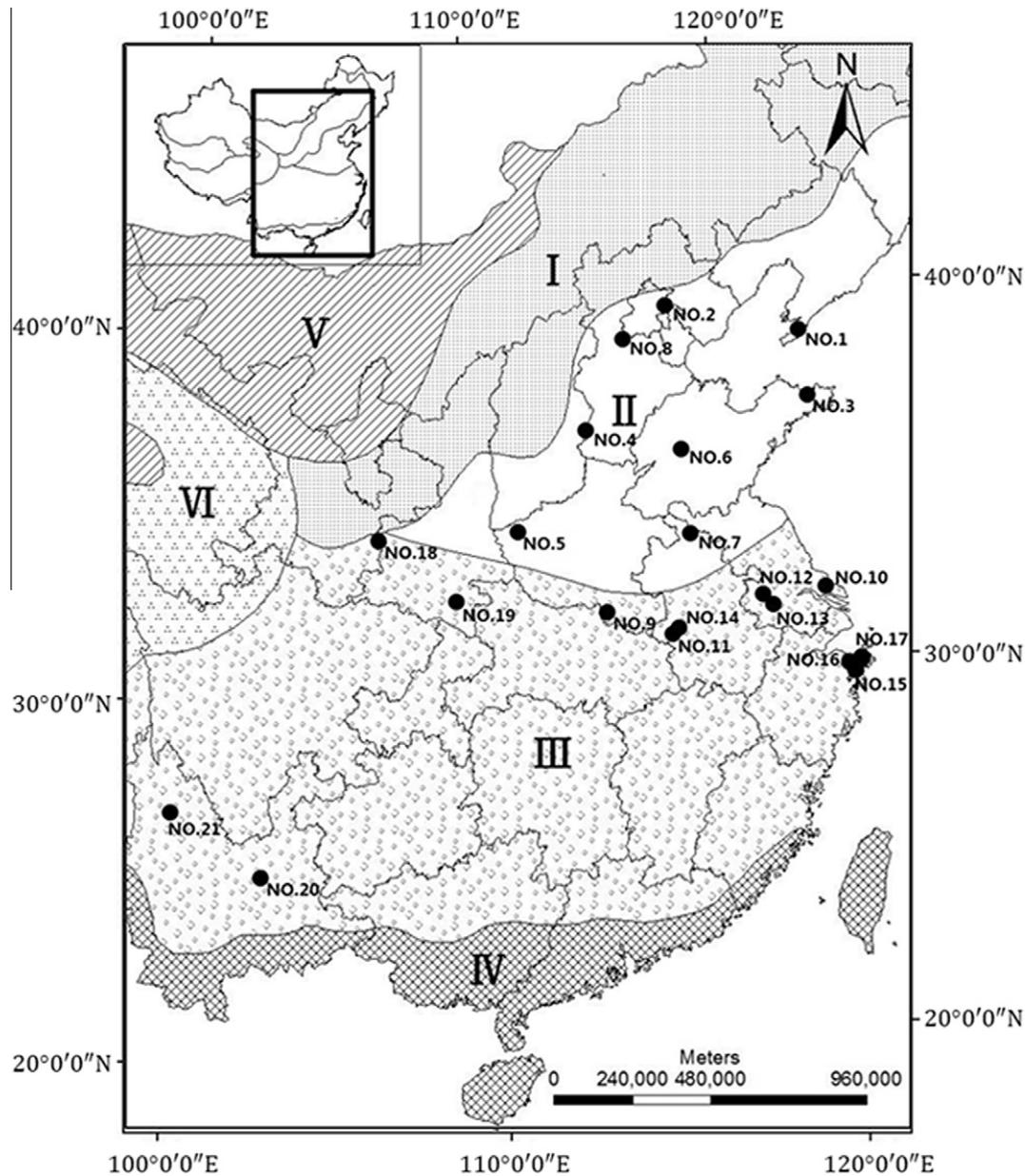


Fig. 1. Distribution map of the oriental oak (*Quercus variabilis*) stands sampled across eastern China. I Temperate grass zone, II temperate broad-leaved deciduous forest zone, III subtropical broad-leaved evergreen forest zone, IV tropical rainforest zone, V temperature desert zone, VI alpine meadow and steppe zone.

115°46'8"W, on the upper temperature gradient) that were divided into eight groups, and homogenized. We extracted fat using chloroform–methanol methods (Daugherty and Lento, 1983), and then measured the C and N content in fat and the remaining biomass.

### 2.3. Climate data

Geographical position and altitude (m) were recorded using a global positioning system (Thales USA). MAT, mean annual precipitation (MAP, mm), average diurnal range of temperature (DRT, °C), and annual precipitation seasonality (coefficient of variation of monthly mean precipitations) (APS, %) were estimated using the global climate dataset with a resolution of  $0.0083 \times 0.0083$  (ca.  $1 \text{ km} \times 1 \text{ km}$ ) obtained from <http://www.worldclim.org/>. Growing season length (GSL, days), was estimated with records of 756 climatic stations in China using spatial interpolation method (during 1954–2007).

### 2.4. Statistical analyses

Elemental concentrations and ratios were  $\log_{10}$ -transformed before analyses to improve the data normality. Linear regression was employed to fit the relationships among the stoichiometric traits (C, N, and P concentrations as well as their ratios) and for climatic (MAT) factors. To overcome the problems caused by collinearity in this study, we used hierarchical partitioning method (Heikkinen et al., 2004). Hierarchical partitioning was conducted using the 'hier.part package' version 0.5-1 (Walsh and MacNally, 2003), which was run as a part of the R statistical package. To determine whether climatic factors affect stoichiometry directly or as mediated through its effects on food quality, a path analysis was conducted to identify the direct effect of climatic factors on weevil larval composition and its indirect effect through food quality. All statistical analyses were performed using SPSS (version 15.0, IBM Corp., Somers, NY, USA) and R 2.2.1 (R Development Core Team 2005).

### 3. Results

#### 3.1. Stoichiometric composition and variation of acorns and weevils

Compared with acorns, the C, N, and P concentrations (expressed in milligram per gram dry weight) in larvae were 39%, 501%, and 270% higher, respectively (Sun et al., 2012; Appendix S1, Table A.1.). Larval C:N and C:P molar ratios were 78% and 63% lower than acorns, and the N:P ratio was 63% higher than in acorns. The concentrations of N (80.8 mg g<sup>-1</sup>) and P (4.6 mg g<sup>-1</sup>) were higher in adults than in larvae (N, 50.7 mg g<sup>-1</sup>; P, 4.32 mg g<sup>-1</sup>) ( $p < 0.0001$ ), but the larvae had significantly higher C concentration (604.8 mg g<sup>-1</sup>) than adults (533.0 mg g<sup>-1</sup>) ( $p < 0.0001$ ) (Appendix S1, Table A.1.). Additionally, there were differences in the chemical compositions of larvae heads and body. Larvae heads were higher in N and P, and lower in C compared to body ( $t$ -test  $p < 0.05$ ) (Appendix S1, Table A.2.).

We observed high variability in larval stoichiometric composition across climatic gradient compare with adults and acorn. Larval N and P concentrations varied from 43.52 to 63.01 mg g<sup>-1</sup> and 3.48 to 5.90 mg g<sup>-1</sup>, respectively, with higher coefficient of variation (CV) (N, CV = 10%; P, CV = 13%) compare to the adults (N, CV = 5%; P, CV = 8%). Similarly, the elemental ratios in larvae also displayed larger variation relative to them in adults (Appendix S1, Table A.1.).

#### 3.2. Weevil stoichiometry related to acorn nutrients and climatic factors

Larval N, P and their ratios were positively associated with corresponding values in acorns. In contrast, C, N and P or ratios in adults showed no significant relationship with corresponding measures in acorns (Appendix S2, Fig. A1), but C, N and P were positively related to larvae C, N and P (Appendix S2, Fig. A2), respectively.

The larval C, N and P stoichiometry were correlated with most of the five climatic factors included in our analysis (MAT, MAP, DRT, APS and GSL, Table 1). Based on hierarchical partitioning (Table 1), most notably, larval C, lipid and P was closely associated with DRT, and N was associated with MAT. The other climatic factors showed close relationships and multicollinearity with MAT and DRT (Appendix S1, Table A.3.). Therefore, we focus the bulk of our analysis on temperature related factors (MAT and DRT) to explain variation in weevil larval stoichiometry. Larval C concentration was positively associated with MAT (C,  $R^2 = 0.30$  and  $p = 0.0103$ ), but N was negatively related to MAT (N,  $R^2 = 0.58$  and  $p < 0.0001$ ) (Fig. 2). Larval C concentration decreased with DRT, while N and P increased with DRT (Fig. 3). Adult stoichiometry displayed no relationship with MAT and DRT (Figs. 2 and 3).

Based on the path analysis, the direct effects of DRT on larval stoichiometry were larger than the indirect effects through affecting the acorn stoichiometry, and the variation in larvae was driven mainly by DRT and MAT (Fig. 4). The path analysis showed that the

variation in larval C and lipid concentrations were driven by DRT, while variation in larva N and P were driven by temperature-related factors and acorn stoichiometry.

#### 3.3. Weevil lipid storage and it related to temperature related factors

Lipid content decreased by >82% between larval (BJ, 172.10 mg g<sup>-1</sup>, HS, 183.64 mg g<sup>-1</sup>, and KM, 178.44 mg g<sup>-1</sup>) and adult (BJ, 23.69 mg g<sup>-1</sup>, HS, 32.63 mg g<sup>-1</sup>, and KM, 30.85 mg g<sup>-1</sup>) stages. Large energy storage lost for fueling metabolism during no food period in the laboratory. The lipid storage in larvae showed some degree of variability, and exhibited consistent significant variation pattern with larval C across the climatic gradient (Fig. 5). Extracted fat were rich in C (768.1 mg g<sup>-1</sup>) and very low in N (below detection range of 0.02–2 mg abs). The C and N in residual larval biomass following lipid extraction were 522.1 mg g<sup>-1</sup> and 85.2 mg g<sup>-1</sup>, respectively, which is similar to the C (532.9 mg g<sup>-1</sup>) and N (80.8 mg g<sup>-1</sup>) concentration in adults (Appendix S2, Fig. A3).

### 4. Discussion

As our expectation, weevil larval stoichiometry and lipid exhibited variability across a climate gradient corresponding to variable requirements for nutrients, and showed closed relationship with temperature-related factors. The emergence adults, by contrast, showed much less stoichiometric variability and no relationship with temperature, likely as this stored energy had been largely consumed. These results indicate that temperature is an important factor driving variation in the stoichiometry of weevil larva at large geographical scale. Overall, these patterns are consistent with an explanation that temperature-associated variation in larval storage of carbon is an important factor in driving stoichiometric variation and weakening stoichiometric regulation in these insect larvae.

#### 4.1. Stoichiometric traits of weevil larvae and adults

Although plants generally have higher C concentration compared to animals (Sterner and Elser, 2002), we found that weevil larvae have a higher C concentration than their food resources because of high lipid storage, implying that needs for energy storage affect patterns of element concentration across trophic levels. We observed that C and N concentrations in residual body mass (after fat was extracted) were similar to C and N concentrations in adults (Appendix S2, Fig. A3). The N and P were relatively conserved during the non-feeding period during which C-rich lipids were consumed as an energy source, resulting in lower C and higher N and P concentrations in adults. Previous studies also have reported a substantial increase in P (almost twice) in last stage copepodites (*Sabinia setosa*) compared with emergence adults (Villar-Argaiz et al., 2002).

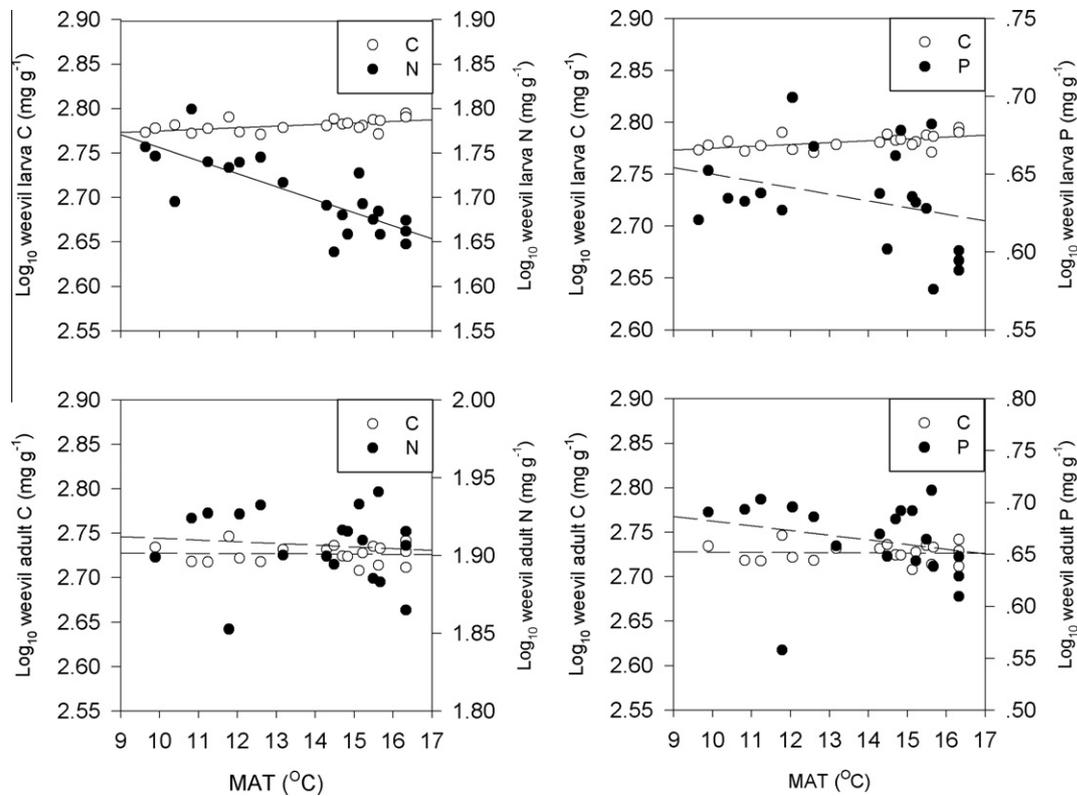
Weevil, as a seed parasite, displayed a lower degree of intraspecific variability in their elemental composition and stoichiometry

**Table 1**

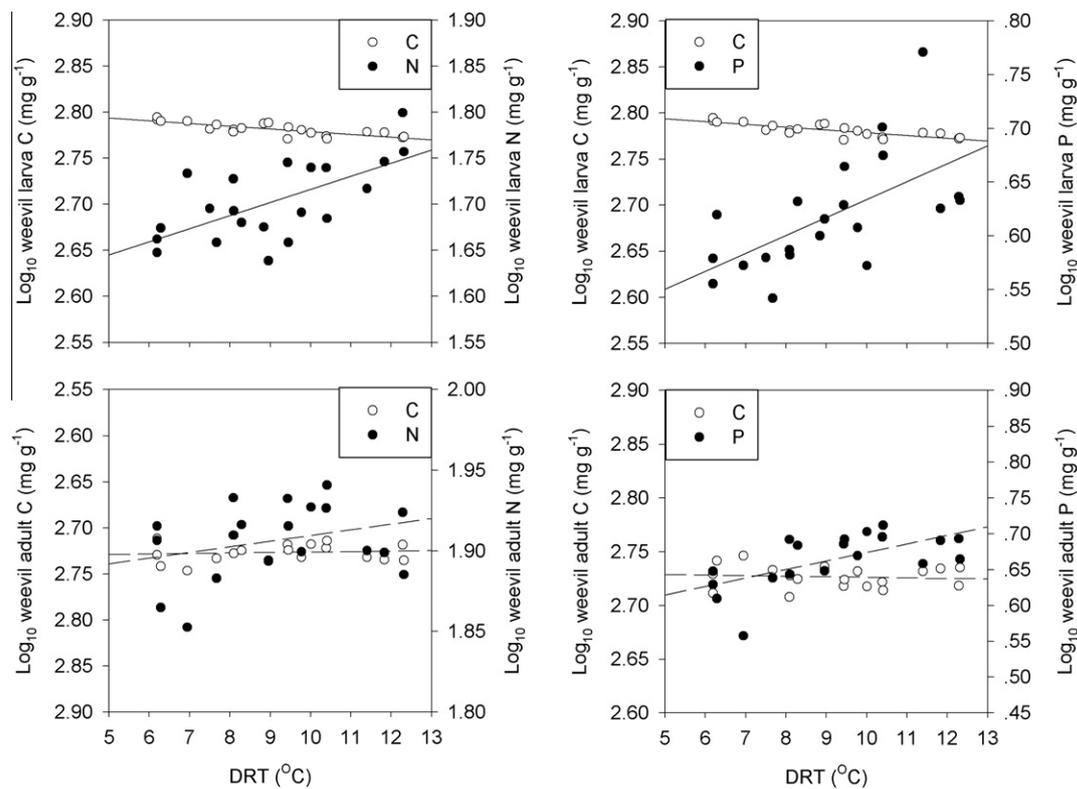
Results of hierarchical partitioning for the effect of climatic factors on soil, and the effect of climatic factors and Mg available on leaf, acorn and weevil Mg.

Larval elements	Full model ( $R^2$ )	Contribution of the individual predictor (%)								
		Climatic factors						Acorn elements (mg g <sup>-1</sup> )		
		MAT	DRT	MAP	ASP	GSL	C	N	P	
C	0.81	9.16	39.67	11.25	13.96	7.15	2.77	7.33	8.71	
N	0.75	22.45	15.86	15.34	14.43	15.96	1.00	14.04	0.92	
P	0.86	5.39	36.99	2.83	7.96	7.80	6.69	19.71	12.64	
Lipid	0.86	8.90	20.22	28.92	17.29	6.68	6.69	5.96	5.34	

Note: See the full methods for the abbreviations.



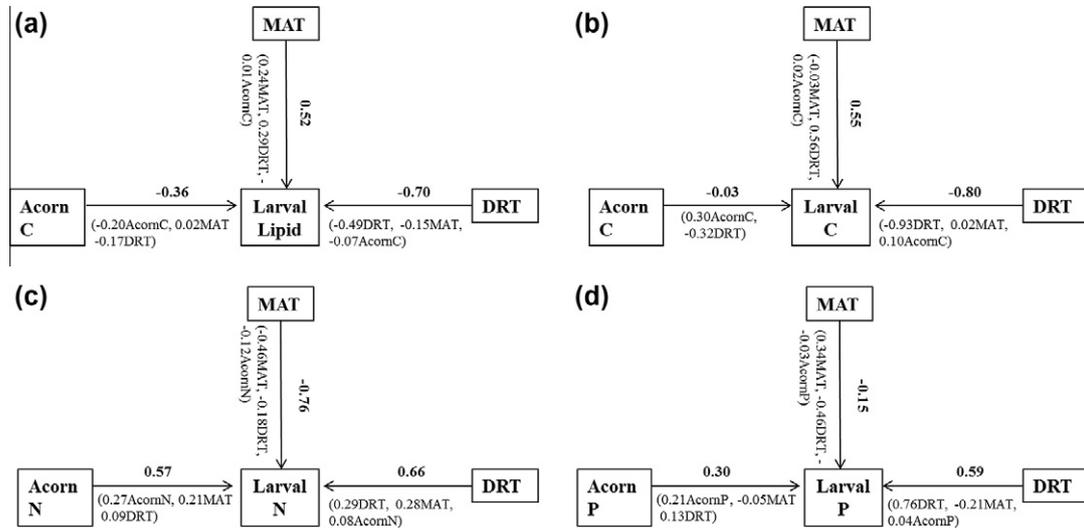
**Fig. 2.** Variations of C, N and P concentrations in weevil larva and adult with MAT (°C). The regression fits were for MAT and C ( $R^2 = 0.30$  and  $p = 0.0103$ ), for MAT and N (acorn,  $R^2 = 0.20$  and  $p = 0.040$ , larvae,  $R^2 = 0.58$  and  $p < 0.0001$ ). The broken line indicates marginal significance.



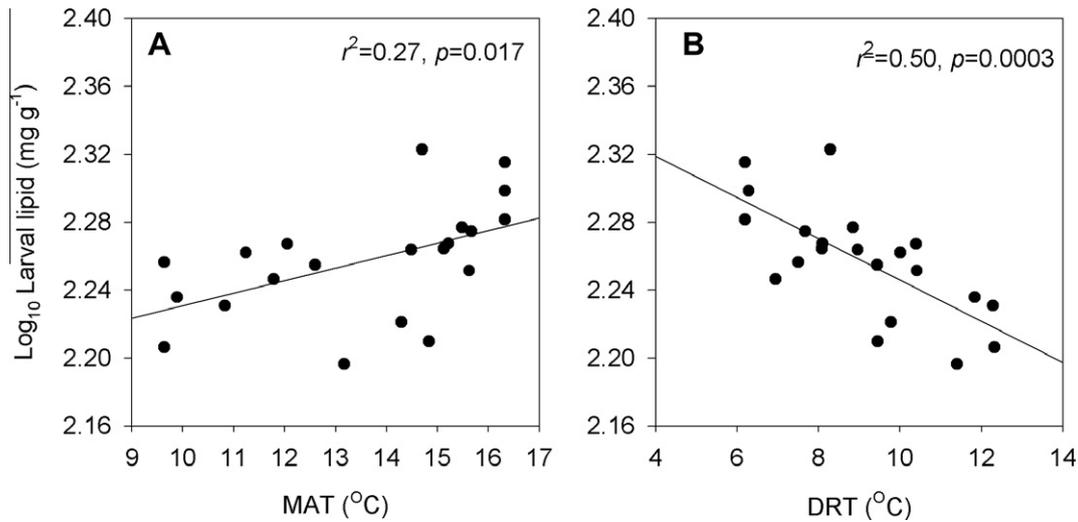
**Fig. 3.** Variations of C, N and P concentrations in weevil larva and adult with DRT (°C). The regression fits were for DRT and larval C ( $R^2 = 0.64$  and  $p < 0.0001$ ), for DRT and larval N ( $R^2 = 0.43$  and  $p = 0.0014$ ), and for DRT and larval P ( $R^2 = 0.37$  and  $p = 0.0033$ ). The broken line indicates marginal significance.

compared with leaf-feeding or insect-feeding consumers reported by other studies. For example, the CV of body C-content in crickets (Bertram et al., 2008) or guppies (El-Sabaawi et al., 2012), is more

than 3 times of the CV of observed here. These differences in intra-specific variation among studies are likely because of the different regulating ability among animals, and different diet stoichiometry



**Fig. 4.** Path analyses of the relationships in C, N, P and lipid concentrations between larval body and environmental factors (acorn, MAT and DRT) in oriental oak stands across eastern China. Numbers in bold type show the Pearson correlation coefficients among the variables, whereas numbers in parentheses partition the Pearson correlation coefficients into direct and indirect effects of MAT and DRT on body C, N and P (i.e. attributable to indirect relationships with the other predictor variable).



**Fig. 5.** The variation patterns of larval lipid concentration with MAT and DRT (°C). The regression fits were for larval lipid and MAT ( $R^2 = 0.27$  and  $p = 0.017$ ), for larval lipid and DRT ( $R^2 = 0.50$  and  $p = 0.0004$ ).

variation and environmental heterogeneity. The low variation observed in body P of adult weevils is low compared to the study of Bertram et al. (2008), which reported that the CV for body P of adult weevils (*S. setosa*) ranged from 16% to 22%. This difference may be due to the lack of feeding for adults sampled here, and the fact that the weevil adults in our study were in the same stage and similar body size. As predicted, the weevil larva displayed high variation relative to weevil adult. One possible interpretation is that the variability produced by shifts in energy storage responds to temperature decreased. These results likely reflect that pupation may play a buffering role in mitigating the effects of temperature and food on adults via transformation from larvae into adults.

**4.2. Variation in weevil elemental concentration with temperature-related factors and food at large spatial scale**

Weevil larvae stoichiometry was related both to temperature-related factors (MAT and DRT) and acorn stoichiometry, but adult stoichiometry was associated only with larvae because they were

rebuilt from larval biomass, and much of the energy storage in larvae had been metabolized.

As observed previously (Kay et al., 2006; Small and Pringle, 2010), our results showed that food is an important factor that drives variation in larvae N and P, with significant increased with acorn N and P. Compared to larval body N and P, body C was affected mainly by temperature related factors, which suggests it depends mainly on physiological acclimation to prevailing temperature. One possible explanation is that, in the warmer region, the weevil larvae contained higher C because of the elevated metabolic demands during diapause in this environment, causing the weevil larvae sequester more fat in order to meet the metabolic demand. The larval body N- and P-content were low in warm region, which is a result produced by interacting effects of acorn properties, organism life strategy and climatic factors. In the T-Plant Physiology Hypothesis (Reich and Oleksyn, 2004), temperature drives variation in leaves stoichiometry by affecting plant biochemistry and physiology, and here we show that temperature could influence weevil larvae stoichiometry directly by regulating biochemical

processes and storage depending on overwintering demand specific to local climate, and also exert an effect on larvae stoichiometry indirectly by altering the composition of its food. The variability in adult elemental composition is likely the product of adults sequestering energy and nutrients at different rates compared to their larval stage, to fulfill the stoichiometric demands of future development and reproduction (Rivero et al., 2001; Telang et al., 2002).

Energy storage is a very common natural phenomenon as consumers commonly experience spatial and temporal variation in resources and other environmental factors, and it has been shown to vary with temperature induced by latitude and altitude (Zhou et al., 1995; Schultz and Conover, 1997). Until now, the contribution of energy storage to stoichiometric variability in consumer stoichiometry has been poorly understood. Our results from this study indicate that energy storage, like food, should be an important factor contributing to the variation in chemical composition of consumers.

## 5. Conclusions

To our knowledge, our study is first to explore the intraspecific stoichiometry in consumers, and the stoichiometric variation pattern across a large geographical scale. This study adds to our understanding of how biological demands for nutrient respond to temperature affect organismal C:N:P. Clearly, future studies need consider the energy storage when explore mechanisms underlying stoichiometric variation in the context of environmental heterogeneity. More experimental studies are necessary to assess the influence of energy storage on variation in organismal stoichiometry and advance the theory of ecological stoichiometry.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jinsphys.2013.01.010>.

## References

- Bertram, S.M., Bowen, M., Kyle, M., Schade, J.D., 2008. Extensive natural intraspecific variation in stoichiometric (C:N:P) composition in two terrestrial insect species. *Journal of Insect Science* 8, 1–7.
- Boswell, A.W., Provin, T., Behmer, S.T., 2008. The relationship between body mass and elemental composition in nymphs of the grasshopper *Schistocerca americana*. *Journal of Orthoptera Research* 17, 1–7.
- Cheng, H.-Y., Hsu, T.-S., 1956. On life history and control measures of the acorn weevil, *Curculio (Balaninus) dentipes* (Roelofs). *Scientia Silvae Sinicae* 1, 68–76.
- Chrzanowski, T.H., Grover, J.P., 2008. Element content of *Pseudomonas fluorescens* varies with growth rate and temperature. *Limnology and Oceanography* 53, 1242–1251.
- Daugherty, C.E., Lento, H.G., 1983. Chloroform–methanol extraction method for determination of fat in foods: collaborative study. *Journal of Association of Official Analytical Chemists* 66, 927–932.
- Denlinger, D.L., 2001. Interrupted development: the impact of temperature on insect diapause. In: Atkinson, D., Thorndyke, M. (Eds.), *Environment and Animal Development: Genes, Life Histories, and Plasticity*. Garland Science Publishing, New York, pp. 235–250.

- El-Sabaawi, R.W., Zandona, E., Kohler, T.J., Marshall, M.C., Moslemi, J.M., Travis, J., et al., 2012. Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy. *Functional Ecology* 26, 666–676.
- Fagan, W.F., Siemann, E., Mitter, C., Denno, R.F., Huberty, A.F., Woods, H.A., Elser, J.J., 2002. Nitrogen in insects: implications for trophic complexity and species diversification. *American Naturalist* 160, 784–802.
- Faerovig, P.J., Hessen, D.O., 2003. Allocation strategies in crustacean stoichiometry: the potential role of phosphorus in the limitation of reproduction. *Freshwater Biology* 48, 1782–1792.
- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science* 293, 2248–2251.
- Hahn, D.A., Denlinger, D.L., 2011. Energetics of insect diapause. *Annual Review of Entomology* 56, 103–121.
- Hahn, D.A., Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology* 53, 760–773.
- Hambäck, P.A., Gilbert, J., Schneider, K., Martinson, H.M., Kolb, G., Fagan, W.F., 2009. Effects of body size, trophic mode and larval habitat on Diptera stoichiometry: a regional comparison. *Oikos* 118, 615–623.
- Han, W., Fang, J., Guo, D., Zhang, Y., 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist* 168, 377–385.
- Han, W.X., Fang, J.Y., Reich, P.B., Woodward, F.I., Wang, Z.H., 2011. Biogeography and variability of 11 mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecology Letters* 14, 788–796.
- Heikkinen, R.K., Luoto, M., Virkkala, R., Rainio, K., 2004. Effects of habitat cover, landscape structure and spatial variables on the abundance of birds in an agricultural forest mosaic. *Journal of Applied Ecology* 41, 824–835.
- Hood, J.M., Sterner, R.W., 2010. Diet mixing: do animals integrate growth or resources across temporal heterogeneity? *American Naturalist* 176, 651–663.
- Kay, A., Rostampour, S., Sterner, R.W., 2006. Ant stoichiometry: elemental homeostasis in stage-structured colonies. *Functional Ecology* 20, 1037–1044.
- Laspoumaderes, C., Modenutti, B., Balseiro, E., 2010. Herbivory versus omnivory: linking homeostasis and elemental imbalance in copepod development. *Journal of Plankton Research* 32, 1573–1582.
- Loladze, I., 2002. Rising atmospheric CO<sub>2</sub> and human nutrition: toward globally imbalanced plant stoichiometry. *Trends in Ecology and Evolution* 17, 457–461.
- Mcfeeters, B.J., Frost, P.C., 2011. Temperature and the effects of elemental food quality on *Daphnia*. *Freshwater Biology* 56, 1447–1455.
- Menu, F., Debouzie, D., 1995. Larval development variation and adult emergence in the chestnut weevil *Curculio elephas* Gyllenhal (Col. Curculionidae). *Journal of Applied Entomology* 119, 279–284.
- Nakazawa, K., 2011. The ontogenetic stoichiometric bottleneck stabilizes herbivore–autotroph dynamics. *Ecological Research* 26, 209–216.
- Nestel, D., Tolmasky, D., Rabossi, A., Quesadaallué, L.A., 2003. Lipid, carbohydrates and protein patterns during metamorphosis of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 96, 237–244.
- Persson, J., Fink, P., Goto, A., Hood, J.M., Jonas, J., Kato, S., 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* 119, 741–751.
- Persson, J., Wojewodzic, M.W., Hessen, D.O., Andersen, T., 2011. Increased risk of phosphorus limitation at higher temperatures for *Daphnia magna*. *Oecologia* 165, 23–29.
- Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences United States of America* 101, 11001–11006.
- Rivero, A., Giron, D., Casas, J., 2001. Lifetime allocation of juvenile and adult resources to egg production in a holometabolous insect. *Proceedings of the Royal Society of London Series B Biological Sciences* 268, 1231–1237.
- Schade, J.D., Kyle, M., Hobbie, S.E., Fagan, W.F., Elser, J.J., 2003. Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecology Letters* 6, 96–101.
- Schultz, E.T., Conover, D.O., 1997. Latitudinal differences in energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). *Oecologia* 109, 516–529.
- Small, G.E., Pringle, C.M., 2010. Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream. *Oecologia* 162, 581–590.
- Sterner, R.W., Elser, J.J., 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.
- Sterner, R.W., Schwalbach, M.S., 2001. Diel integration of food quality by *Daphnia*: luxury consumption by a freshwater planktonic herbivore. *Limnology and Oceanography* 46, 410–416.
- Sun, X., Kang, H., Du, H., Hu, H., Zhou, J., Zhou, J., et al., 2012. Stoichiometric traits of oriental oak (*Quercus variabilis*) acorns and their variations in relation to environmental variables across temperate to subtropical China. *Ecological Research*. <http://dx.doi.org/10.1007/s11284-012-0953-5>.
- Tao, L., Hunter, M.D., 2012. Does anthropogenic nitrogen deposition induce phosphorus limitation in herbivorous insects? *Global Change Biology*. <http://dx.doi.org/10.1111/j.1365-2486.2012.02645.x>.
- Telang, A., Buck, N.A., Wheeler, D.E., 2002. Response of storage protein levels to variation in dietary protein levels. *Journal of Insect Physiology* 48, 1021–1029.
- Ventura, M., Catalan, J., 2005. Reproduction as one of the main causes of temporal variability in the elemental composition of zooplankton. *Limnology and Oceanography* 50, 2043–2056.

- Villar-Argaiz, M., Medina-Sánchez, J.M., Carillo, P., 2002. Linking life history strategies and ontogeny in crustacean zooplankton: implications for homeostasis. *Ecology* 83, 1899–1914.
- Walsh, C., MacNally, R., 2003. Hierarchical Partitioning, R Project for Statistical Computing, <<http://cran.r-project.org>>.
- Wang, J., Wang, S.B., Kang, H.Z., Xin, Z.J., Qian, Z.H., Liu, C.J., 2009. Distribution pattern of oriental oak (*Quercus variabilis* Blume) and the characteristics of climate in eastern Asia. *Journal of Shanghai Jiaotong University* 27, 235–241.
- Zhou, Z., Honek, A., Powell, W., Carter, N., 1995. Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. *Entomologica Experimentalis et Applicata* 75, 99–107.
- Yu, Q.A., Chen, Q.S., Elser, J.J., He, N.P., Wu, H.H., Zhang, G.M., Wu, J.G., Bai, Y.F., Han, X.G., 2010. Linking stoichiometric homeostasis with ecosystem structure, functioning and stability. *Ecology Letters* 13, 1390–1399.