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Allometric and phylogenetic variation in insect phosphorus content

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Summary

1. Phosphorus content was measured in adult insects and arachnids from 170 species collected in the Sonoran Desert.

2. Across insect body sizes spanning four orders of magnitude, phosphorus content was inversely related to body mass. The largest species (~1 g dry) had phosphorus contents that were only about 60% (0·62% P absolute) as high as phosphorus contents of the smallest species (~0·0001 g dry; 0·97% P). Negative phosphorus allometry was observed within each of seven insect orders and within arachnids.

3. Phosphorus contents of insect predators and herbivores were statistically indistinguishable.

4. More recently derived orders tended to have lower phosphorus contents – with the exception of the most recently derived group (Panorpida = Diptera + Lepidoptera), which had high phosphorus contents.

Key-words: Allometry, body size, exoskeleton, phosphorus limitation, stoichiometry

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Introduction

Herbivorous insects face a fundamental asymmetry between the nutrient contents of their body tissues and those of the plants they eat (Slansky & Feeny 1977; McNeill & Southwood 1978; Mattson 1980; Strong, Lawton & Southwood 1984; White 1993; Elser *et al*. 2000a). Research in this area has focused primarily on nitrogen (N) as a limiting nutrient. Recently, however, Elser *et al*. (2000a) demonstrated that the elemental profiles of terrestrial plants (photosynthetic tissues) are sufficiently poor in phosphorus (P) relative to carbon (C) and N, that P may often limit terrestrial herbivores, much as it does in aquatic ecosystems (Elser *et al*. 2000a; Sterner & Schulz 1998). This prediction is supported by several recent studies demonstrating possible P-limitation in terrestrial insects (Perkins 2001; Woods *et al*. 2002; Schade *et al*. 2003).

Here we use a large database to analyse the effects of three factors – trophic level, body size and taxonomy – on insect P content. Previous studies found that each factor contributes to variation in N content among insect species (Siemann *et al*. 1996; Fagan *et al*. 2002a). No similarly broad analyses have been conducted for insect P content. However, understanding macro-patterns in insect P content is important, both because insects play fundamental roles in ecological processes dependent on elemental composition (e.g. nutrient cycling) and because P occupies a key position in the recently developed theory of ecological stoichiometry (Sterner & Elser 2002).

Several expectations can be derived *a priori* by extensions of previous work on N. First, like N (Fagan *et al*. 2002a), P should be more concentrated in predaceous than herbivorous insects. Although no studies have yet examined this possibility, insect P content does appear to be influenced by dietary P availability, in both natural (Schade *et al*. 2003) and laboratory (Woods *et al*. 2002) settings. Second, more derived lineages may contain less P. Fagan *et al*. (2002a), for example, found that among terrestrial herbivorous insects, the most ancient lineage analysed (Orthoptera) contained significantly more N per unit body mass than did the most derived lineage (the Panorpida, parent to the Diptera and Lepidoptera). In addition, Jaenike & Markow (2003) found a strong positive correlation (interspecific) between N and P contents of diverse species of *Drosophila*.

Third, P content may be only weakly related to body mass, as N appears to be (Fagan *et al*. 2002a). This expectation, however, is qualified by other observations. Several taxa exhibit inverse dependence of P content on body size (e.g. plants; Nielsen *et al*. 1996). Moreover, ecological stoichiometry provides a theoretical basis for expecting such inverse relationships (Reiners 1986; Elser *et al*. 1996, 2000b; Sterner & Elser 2002). Specifically, Elser *et al*. (1996) suggested (the

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Table 1. References used to construct composite phylogenetic trees (trees available at http://lsvl.la.asu.edu/irceb/stoichiometry/insectPdata.html)

Taxon	References	Number of species
Orthoptera	Chapco, Martel & Kuperus (1997); Otte & Nesrecki (1997)	14
Hemiptera	Carver, Gross & Woodward (1991); Schuh (1979); Schuh & Slater (1995); Wheeler, Schuh & Bang (1993)	30
Coleoptera	Kukalová-Peck & Lawrence (1993); Lawrence & Newton (1982)	37
Hymenoptera	Ascher, Danforth & Shuqing (2001); Ronquist *et al*. (1999)	19
Diptera	McAlpine (1989); Wiegmann, Mitter & Thompson (1993); Wood & Borkent (1989); Yeates & Wiegmann (1999)	21
Lepidoptera	Lemaire & Minet (1999); Pollack *et al*. (1998); Weller, Pashley & Martin (1996)	12

growth rate hypothesis) that rapid growth requires disproportionate investment in synthetic cellular components, especially ribosomes. Ribosomes often contain a large fraction of total cellular RNA (as rRNA), which is particularly P-rich. Therefore, rapidly growing, rRNA-rich organisms should exhibit a distinct high-P signature (see Woods *et al*. 2003). Because most taxonomic groups exhibit inverse allometry of mass-specific growth rates (Peters 1983), P content of insects should scale inversely with body size.

Overall we find mixed support for these predictions, with available data suggesting influences of body size and phylogeny but not trophic level on insect P content.

Materials and methods

Adult insects and arachnids were collected in the Sonoran Desert, near Phoenix, Arizona (USA) during the summer of 2000. Additional, smaller collections were made in the Chiricahua Mountains (Southwest Research Station) in south-eastern Arizona. Samples were cooled on ice, returned to Arizona State University, and frozen until used. Before analysis, individuals were identified at least to family and usually to genus and species and were then dried to a constant weight at 55 °C. Number of individuals per species ranged from 1 to 18 (mean 2·4 ± 0·2). No attempt was made to distinguish sex.

Phosphorus content (%P, dry mass basis) was assayed using persulphate digestion and ascorbate–molybdate colorimetry, as described by Woods *et al*. (2002). Samples were prepared for P analysis in one of two ways. Individuals larger than about 10 mg were ground in a mortar into fine particles, which were subsequently dried again. Subsamples (0·5–2 mg; weighed on a Mettler MT5 balance, ±0·1 μg) were then assayed for P content. Individual insects smaller than 10 mg (dry) were assayed whole. These specimens were placed at the bottom of a digestion tube and lightly crushed with a Teflon-tipped rod before proceeding with the assay. For very small specimens (<200–300 μg), we performed the persulphate digestion in smaller tubes containing reduced volumes of liquid. Because of our interest in the effects of body size on P content, we performed extensive testing using groups or individuals from an inbred population of adult *Drosophila melanogaster* (approximately 0·4 mg dry mass per fly) to ensure that the two processing methods did not give systematically different results (they did not).

Using a large database on insect N content compiled from the literature, Fagan *et al*. (2002a) found that predators had significantly higher N content than did herbivores. We thus began by examining our phosphorus data for patterns related to feeding mode. Trophic mode is highly conserved across insect groups and therefore is best analysed in a phylogenetic framework. We mapped trophic level and body P content onto composite trees assembled from the current literature (for 133 species; references are given in Table 1; see http://lsvl.la.asu.edu/irceb/stoichiometry/insectPdata.html for phylogenies and raw data). Locations of shifts in trophic level were identified by parsimony optimization. We then divided the trees into a maximal number of non-overlapping contrasts, each consisting of a set of contiguous branches containing an inferred evolutionary shift in trophic level (Fagan *et al*. 2002a). Table 2 shows taxonomic groups used for contrasts. The values of P content and log body mass used for contrasts were the inferred ancestral value for each group – obtained by working backwards through nodes and calculating the %P and log mass at each older node as the median value of %P in all taxa stemming from the node. Contrasts in P content were then regressed against contrasts in log body mass. This test provides a phylogenetically rigorous test for effects of trophic level, while simultaneously controlling for body size.

The analysis above showed that trophic level had no effect on P content (see Results). We therefore analysed the remaining data without regard to feeding mode. The effects of body size were analysed by standard regression analysis and by accounting for phylogenetic relationships among taxa. For standard regressions, we treated mean values for species data as independent and used least squares linear regression (the error in our estimates of log body size was probably much smaller than the error in body phosphorus content). In this analysis we included all species in the database (170 species) regardless of feeding mode (even when the mode was unknown), including 15 species of arachnids.

Table 2. Composition of independent contrasts used to test for effect of trophic level (of adults) on body P content

Ordinal group and predator taxa	Herbivore taxa	No. of predators in contrast	No. of herbivores in contrast
Paleoptera	Orthoptera	5	14
Panorpida			
Asilidae	Bombyliidae	2	4
Dolichopodidae + Tachinidae	Lepidoptera	5	12
Hemiptera			
Nabidae + Anthocoridae	Miridae + Tingidae	2	11
Veliidae	Rhopalidae	3	1
Geocoris	*Lygaeus*	2	1
Hymenoptera			
Pompilidae	Halictidae + Megachilidae + Apidae	3	7
Coleoptera			
Hydrophilidae	Staphylinidae	1	1
Lampyridae + Cantharidae + Lycidae	Elateridae	6	1
Cleridae + Melyridae	Curculionidae + Chrysomelidae	3	4
Adephaga (Carabidae + Dytiscidae)	Scarabaeidae	6	6

We used two methods to account for relatedness among species. The first was to perform regressions of P content against log body mass *within* each order. Although this approach treats species within orders as statistically independent, regardless of their actual phylogenetic relationships, it does provide independent replication at the level of order (diversification of P content and body size in a particular order is independent of diversification in another). Common responses across orders that match the overall result would suggest that the overall result is not generated by unusual order-specific patterns of P content and size.

As a second approach, we used the composite trees described above to perform more rigorous corrections for relatedness, excluding species not identifiable as predaceous or herbivorous (a subset of those used in the standard regression analysis described above). This approach is conservative in that it avoids using species from unknown or underrepresented trophic groups possibly having very different P content. Ideally we would estimate ancestral trait values using information on branch lengths. However, branch length data were available for only limited portions of the phylogenies. Consequently, following the concept of independent contrasts (Felsenstein 1985), we employed a distance measure that quantifies phylogenetic structure among species by counting how many nodes separate each pair of taxa (e.g. Fagan *et al*. 2002b). Phylogenetic data and trait values were used to build matrices of interspecific distance in units of log body mass and P content. We then performed a series of partial Mantel tests (Smouse, Long & Sokal 1986) using the software package PASSAGE (Rosenberg 2001) to explore the significance of correlations between matrices of log body mass and P content while holding phylogenetic relatedness constant across taxa.

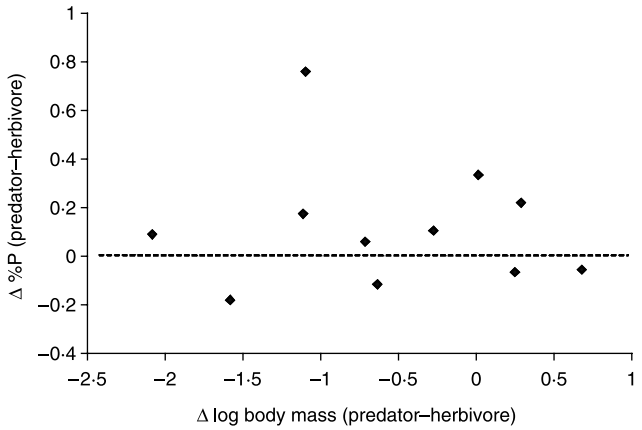

Fig. 1. Independent contrasts between predator and herbivore P content, controlling for contrasts in body size (contrasts identified in Table 2). The dashed line marks the null expectation across body sizes if predator and herbivore P contents do not differ. Least squares regression fitted to the data gives the equation: contrast in P = 0·11 − 0·022 × (contrast in body size). Neither slope nor intercept was significantly different from zero, indicating that trophic feeding style does not affect insect P content.

Results

We obtained P contents for adults of 170 species distributed across eight major taxonomic groups (Arachnida, Odonata, Orthoptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera and Diptera). Mean (± SEM) phosphorus content for all species was 0·791 ± 0·017% P.

Altogether we identified 11 independent shifts between predaceous and herbivorous feeding modes (Table 2), distributed fairly evenly across insect orders. The contrasts analysis reveals that predaceous insects did not have significantly higher P content than did herbivorous insects (Fig. 1) and that this was true across a range of contrasts in body size.

A linear regression fitting P content as a function of log body mass and ordinal group showed significant effects of both factors (Table 3), with log body mass accounting for more of the variation. The coefficients for log body mass extracted from the fitted model gives the equation:

Table 3. ANOVA summary of the effects of body mass and order on P content

Source of variation	d.f.	Mean square	*F*
Log mass	1	1·32	36·3***
Order	7	0·18	4·8***
Log mass × Order	7	0·02	0·6
Residuals	155	0·04	

***$P < 0{\cdot}001$.

$$\%P = 0{\cdot}624 - 0{\cdot}086 \times \log \text{body mass}. \quad \text{eqn 1}$$

Across the range of body sizes in this study (approximately four orders of magnitude; see Fig. 2), the fitted model indicates that the largest species (~1 g dry) had only about 60% the P content (per dry mass) of the smallest species (~100 μg).

The fitted regression model also provides contrast coefficients for each ordinal group, which describe the vertical offset of the group's position from the overall regression line (because the interaction term was non-significant, we treat each ordinal group's slope as identical to the overall fitted slope, –0·086). The coefficients provide a means of comparing P content across orders at a common body size. We picked the common size of 10 mg ($\log_{10}0{\cdot}01 = -2$) because all ordinal groups except Orthoptera include this size (Fig. 2). Arachnida have significantly higher P content than Coleoptera and Hymenoptera, and the Panorpida (Lepidoptera + Diptera) have significantly higher P content than Hymenoptera (Fig. 3).

The regression analyses above incorrectly treat the data as statistically independent. We therefore used two other methods to explore the effect of phylogeny on the relationship between body size and P content. First, we fitted regression lines to each order separately. P content was inversely related to body size in all eight groups (Fig. 2 and Table 4; binomial probability of all eight slopes being negative by chance = 0·004). Of the eight individual slopes, three were significantly negative (Arachnida, Hemiptera, Hymenoptera) and two were marginally significant (Coleoptera, Orthoptera).

Partial Mantel tests of phylogenetically corrected data (132 species) consistently supported the inverse relationship between body size and P content (Table 5). All seven correlations were negative (two of them contained Orthoptera so that there were six independent correlations; probability that all six were negative by chance is 0·016). Of these seven, four were significantly negative.

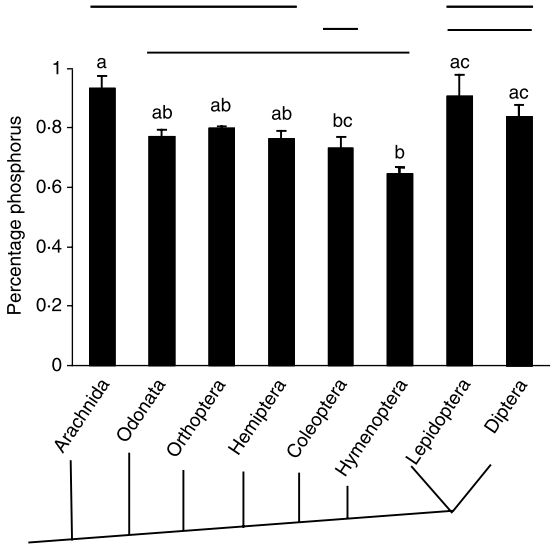

Fig. 3. Size-adjusted phosphorus content (dry mass basis) for the eight major taxonomic groups in the database (mean ± SEM). Error bars for these values were calculated from residuals within each order from the overall fitted regression model. Tukey HSD was used for post-hoc analysis of pairwise differences between size-adjusted values. Taxa denoted by the same letter were not significantly different ($\alpha = 0{\cdot}05$). Horizontal lines provide another way of visualizing significance relationships; lines at the same height denote taxa that were not significantly different.

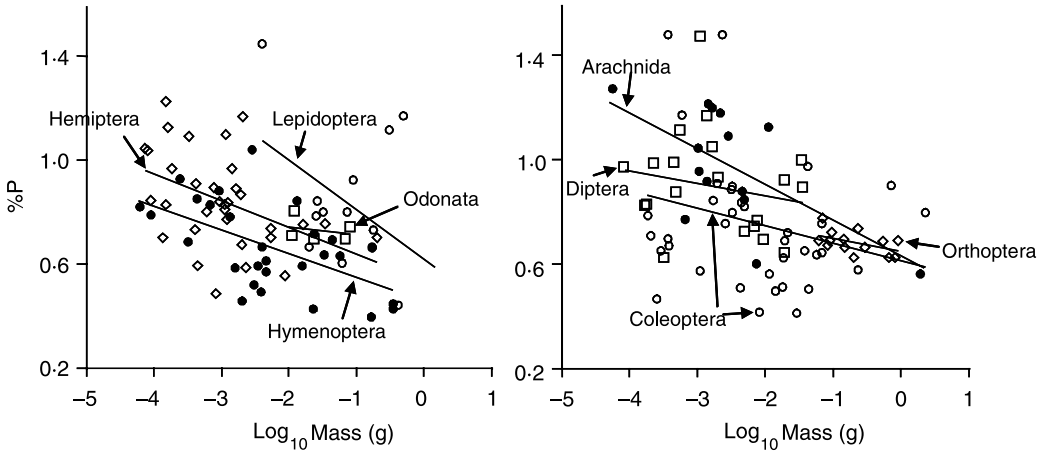

Fig. 2. Semi-log plot of total body %P (by dry mass) as a function of log body mass for 170 species (155 insect species in seven orders, and 15 arachnids). Lines represent least squares fits within each group. See Table 4 for regression coefficients and associated statistics. Table 3 gives ANOVA statistics for the overall fitted regression model.

Table 4. Within-order and overall regression statistics for %P as a function of log body mass

Taxonomic group	*N*	Intercept	Slope	*P*
Arachnida	15	0·63 ± 0·13	−0·138 ± 0·047	0·012
Coleoptera	39	0·59 ± 0·10	−0·086 ± 0·044	0·061
Diptera	22	0·79 ± 0·13	−0·028 ± 0·052	0·393
Hemiptera	32	0·52 ± 0·14	−0·109 ± 0·043	0·013
Hymenoptera	30	0·45 ± 0·08	−0·092 ± 0·043	0·001
Lepidoptera	13	0·62 ± 0·14	−0·189 ± 0·114	0·126
Odonata	5	0·68 ± 0·10	−0·034 ± 0·062	0·628
Orthoptera	14	0·65 ± 0·02	−0·035 ± 0·024	0·072
All species together	170	0·62 ± 0·04	−0·079 ± 0·014	<0·001

Table 5. Summary of correlation coefficients for phylogenetically corrected associations between log body mass and %P

Taxon	Correlation	*P*
Orthoptera + Odonata	−0·253	0·047
Orthoptera (alone)	−0·073	0·538
Hemiptera	−0·789	<0·001
Coleoptera	−0·003	0·951
Hymenoptera	−0·299	0·013
Lepidoptera	−0·598	0·001
Diptera	−0·051	0·628

Discussion

Analyses of adult insects from the Sonoran Desert indicated a significant inverse dependence of P content on body size. The size dependence of P is consistent with relationships found in other taxa (Nielsen *et al*. 1996). It also supports a key prediction of the growth rate hypothesis (Elser *et al*. 1996): that small, fast-growing organisms should exhibit a distinct, high-P signature. We also found variation among taxonomic groups, but no effect of trophic level. Restricting our analysis to a single developmental stage (adult insects) and to a single biogeographical region (the Sonoran Desert) bypasses methodological pitfalls that complicated interpretations of trophic, phylogenetic and allometric signals in insect N content in the study by Fagan *et al*. (2002a). However, our spatially focused data set also requires that caution be exercised when extrapolating to other biomes. In particular, desert soils typically are P-rich compared with those in other ecosystem types, and, in the one data set in which it has been studied, both plant and insect P were positively correlated with soil P (Schade *et al*. 2003). Consequently, the measured organismal P contents in our data set may be higher than those that would be obtained with organisms collected in areas containing more mesic or weathered soils.

INSECT BODY SIZE AND PHOSPHORUS CONTENT

One factor that probably contributes to P allometry is size-dependent shifts in the composition of metabolically active cellular contents, which in organisms adapted for more rapid growth should contain higher concentrations of P-rich biosynthetic molecules (rRNA; Elser *et al*. 1996). Because smaller organisms usually have higher mass-specific growth rates (Peters 1983), P contents should decrease with body size (Fig. 2). A difficulty with this reasoning, however, is that we analysed P content as a function of adult body size. Although many adult insects do grow, many do not, and in most species growth occurs primarily in larval or nymphal stages. Allometry of adult P content could nonetheless stem from functional linkages between P content and growth rate, if the following three relationships hold true:

1. Smaller juvenile insects grow (on a mass-specific basis) more rapidly than larger juveniles (some evidence for this exists at the interspecific (Peters 1983) and ontogenetic levels; Slansky & Scriber 1985).
2. Fast-growing immatures have higher P content (no interspecific support, but ontogenetic: Church & Robertston 1966; T. Watts & T.A. Markow, personal communication).
3. Adult and juvenile P contents are correlated. No data are available on cross-stage correlations in P content, but Fagan *et al*. (2002a) found a strong intraspecific correlation (positive) between the N contents of adult and juvenile insects.

A second mechanism likely to drive P allometry involves changes in allocation to structural material (Schmidt-Nielsen 1984; Reiners 1986). If exoskeletons scale to body mass with an exponent greater than 1, and if insect cuticle has systematically lower P content than does soft tissue, smaller insects should exhibit higher body %P. Little is known about scaling of exoskeletons. However, Prange (1977) argues that arthropod exoskeletons should scale to body mass with an exponent between 1·0 and 1·17, and Anderson, Rahn & Prange (1979) found that across ontogeny exoskeleton mass scaled to body mass (in three spider species) with an exponent of 1·12. Scaling of exoskeleton mass *per se* will lead to scaling of P content only if exoskeletons and soft tissues contain different fractions of P. Barring some exceptions (Gilby & McKellar 1976), however, insect cuticular P content does appear to be very low (Hackman 1984). Other potential structural explanations include size-related changes in the effects of diet on head size (Bernays 1986) or allometric variation in allocation to high-P reproductive tissue (Markow, Coppla & Watts 2001). A more mechanistic understanding of P allometry will require data on P allocation among tissues and relative tissue masses spanning a range of insect body sizes.

Negative allometry of P content suggests that, under natural ecological circumstances, small insects may be more likely than large insects to experience P-limited growth, all else being equal. Few studies have examined this possibility, although recent data suggest that P limitation may occur in caterpillars of *Manduca*

sexta (Perkins 2001; Woods *et al*. 2002), the weevil *Sabinia setosa* (Schade *et al*. 2003) and diverse species of *Drosophila* (Jaenike & Markow 2003). Interestingly, larval *M. sexta* are comparatively enormous among insects (up to 10 g fresh) – if smaller insects are more likely to be P-limited, P-limitation may be very common among insects indeed (Elser *et al*. 2000a). This hypothesis is of course subject to important caveats. The strongest apparent objection – current lack of support in the nutritional ecology literature – represents primarily a lack of tests rather than active rejection. However, small insects may be better able to exploit spatial heterogeneity to select P-rich foods. In addition, the likelihood of P-limitation in any particular taxon is a function of both P content of its food and the extractive ability of its physiology; small insects may avoid potential P problems as a consequence of evolutionary shifts in feeding and digestive physiology.

NO EFFECT OF TROPHIC LEVEL

Fagan *et al*. (2002a) found a strong effect of trophic level on insect N content: predators had on average 14–20% (relative) more N than herbivores. Our study, in contrast, showed that P contents of predators and herbivores were not significantly different (Fig. 1). A possible explanation is that herbivores and predators arrive at roughly the same body P content as a consequence of alternative feeding strategies that deliver roughly the same amounts of P *per time* (but not per unit food consumed). Although herbivores in general consume P-poor food, they often consume great quantities of it. Predators eat P-rich food, but often do so infrequently. The two strategies may therefore represent alternative outcomes of a quantity–quality tradeoff that result in similar body P contents. Additional work should examine why trophic patterns in N and P content differ.

EVOLUTIONARY PATTERNS OF PHOSPHORUS CONTENT

Fagan *et al*. (2002a) found that, among terrestrial herbivorous insects, a recently derived group (the Panorpida, containing Diptera and Lepidoptera), exhibited lower body N contents, on average, than did more ancestral groups. In contrast, we found here that the Panorpida had the highest P content of any of the insect groups. This pattern appears robust, as it is supported by additional data on insect P content culled from the literature as part of the analysis of body N content by Fagan *et al*. (2002a). The data are heterogeneous with respect to body size, developmental stage, biogeographical area and phylogenetic group, but ANOVA nonetheless indicates significantly more %P in members of the Panorpida ($0{\cdot}98 \pm 0{\cdot}07$; $N = 9$) than for other insects ($0{\cdot}76 \pm 0{\cdot}06$, $N = 17$) ($F_{1,24} = 4{\cdot}26$, $P = 0{\cdot}03$).

Physiological processes responsible for the high P content are unknown. When feeding on P-rich food in the laboratory, larval Lepidoptera can develop high levels of organic phosphate in the haemolymph (Wyatt 1961; Woods *et al*. 2002), although the levels of P in the haemolymph of larvae feeding under more natural circumstances are unknown. Evolutionary processes leading to elevated P content among Lepidoptera and Diptera also are unknown. We speculate that they exploit, in general, more ephemeral resources requiring higher rates of larval growth and disproportionate investment in P-rich biochemistry (Elser *et al*. 1996). Another possibility is that fecundity of panorpidans is unusually high relative to other insects and that high P content reflects disproportionate investment in biosynthetically active reproductive tissues.

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