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Exoskeletal Chitin Scales Isometrically With Body Size in Terrestrial Insects

Hilary M. Lease[1,2]* and Blair O. Wolf[1]

[1]*Biology Department, University of New Mexico, Albuquerque, New Mexico 87131*
[2] *Biology Department, Whitman College, Walla Walla, Washington 99362*

ABSTRACT The skeletal system of animals provides the support for a variety of activities and functions. For animals such as mammals, which have endoskeletons, research has shown that skeletal investment (mass) scales with body mass to the 1.1 power. In this study, we ask how exoskeletal investment in insects scales with body mass. We measured the body mass and mass of exoskeletal chitin of 551 adult terrestrial insects of 245 species, with dry masses ranging from 0.0001 to 2.41 g (0.0002–6.13 g wet mass) to assess the allometry of exoskeletal investment. Our results showed that exoskeletal chitin mass scales isometrically with dry body mass across the Insecta as $M_{chitin} = a M_{dry}^{b}$, where $b = 1.03 \pm 0.04$, indicating that both large and small terrestrial insects allocate a similar fraction of their body mass to chitin. This isometric chitin-scaling relationship was also evident at the taxonomic level of order, for all insect orders except Coleoptera. We additionally found that the relative exoskeletal chitin investment, indexed by the coefficient, a, varies with insect life history and phylogeny. Exoskeletal chitin mass tends to be proportionally less and to increase at a lower rate with mass in flying than in nonflying insects ($M_{flying\ insect\ chitin} = -0.56 \times M_{dry}^{0.97}$; $M_{nonflying\ insect\ chitin} = -0.55 \times M_{dry}^{1.03}$), and to vary with insect order. Isometric scaling ($b = 1$) of insect exoskeletal chitin suggests that the exoskeleton in insects scales differently than support structures of most other organisms, which have a positive allometry ($b > 1$) (e.g., vertebrate endoskeleton, tree secondary tissue). The isometric pattern that we document here additionally suggests that exoskeletal investment may not be the primary limit on insect body size. J. Morphol. 271:759–768, 2010.

KEY WORDS: allometry; exoskeleton; scaling; body size; insects; cuticle; support structures; skeleton; chitin

INTRODUCTION

Skeletal systems provide the structural support for movements and other activities that are vital to an animal's survival. Skeletons are mechanical structures that can be internal, external, or hydrostatic, and overinvestment in skeletal systems relative to other organ systems has the potential to constrain animal function and body size (McMahon and Bonner, 1983; Schmidt-Nielsen, 1984). Arthropods have exoskeletons, which have functions similar to those of vertebrate endoskeletons: they support the weight of the animal, provide sites for muscle attachment to enable locomotion, and additionally provide protection from the environment.

Many anatomical and physiologic characteristics of animals are directly related to body size (McMahon and Bonner, 1983; Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Brown and West, 2000). Measuring variation in an animal trait over a wide range of body sizes produces an allometric relationship, which has the mathematical form: $Y = a \times M^{b}$ (where Y is a dependent variable, a is a normalization constant, M is a body mass, and b is the scaling exponent). When $b > 1$, Y increases faster than body mass (positive allometry), when $b < 1$, Y increases less rapidly than mass (negative allometry), and when $b = 1$, Y is linearly proportional to mass (isometry). The scaling of skeletal systems in vertebrates has been studied in diverse taxa (Prange and Christman, 1976; Prange et al., 1979; Miller and Birchard, 2005), and results show that the endoskeleton in most vertebrates scales with positive allometry ($b > 1$). The relationship between exoskeletal mass and body mass in arthropods, in contrast, has received very little attention. For arthropods, work is limited to an analysis by Anderson et al. (1979) who measured the exoskeletal mass in three species of spiders and found that exoskeleton also scales with positive allometry ($b = 1.14$), and an analysis by Wheatly and Ayers (1995) who measured the mineral content of one species of crayfish and found that scaling exponents varied from negative to positive allometry ($b = 0.93$ to $b = 1.27$).

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*Correspondence to: Hilary M. Lease, Biology Department, University of New Mexico, Albuquerque, NM 87131.
E-mail: hlease@unm.edu

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To our knowledge, no studies have examined the relationship between exoskeletal mass and body mass across a large range of arthropod taxa. Understanding how exoskeletal investment scales with body mass is important because the relative investment in one structure may limit or constrain animal performance or the potential allocation to other important structures. In this study, we use the chitinous portion of the procuticle as a measure of exoskeletal mass (see rationale later). We measure the mass of exoskeletal chitin in 245 species of insects, with representatives from a range of body sizes and life styles, and examine how the exoskeletal chitin mass varies with body mass. Our sample includes species from 15 orders and 91 families of insects. We use these data to address two questions: (1) how does exoskeletal chitin scale with body size in terrestrial insects? and (2) how do phylogeny and lifestyle interact to affect the allometry of exoskeletal chitin investment?

Structure of the Exoskeleton

Functionally, the exoskeleton (the cuticle) provides the arthropod body with its shape and rigidity. The general structure of the exoskeleton is well conserved across arthropod taxa (Brown, 1975; Hadley, 1985), and in all arthropods, the cuticle is a multilayered structure (epicuticle, exocuticle, and endocuticle) that is made primarily of chitin, proteins, and lipids (Brown, 1975; Hadley, 1985). The outer layer or epicuticle is composed of lipoproteins, is extremely thin (Barbakadze et al., 2006) and inelastic (Brown, 1975), and functions as a permeability barrier (Andersen, 1979). The procuticle (exocuticle + endocuticle), in contrast, is primarily made of a protein-chitin complex, makes up the majority of the total cuticular thickness, and provides structural integrity to the exoskeleton (Brown, 1975; Andersen, 1979; Hadley, 1985). Chitin is the major polysaccharide in the cuticle (Kramer et al., 1995) and usually makes up between 20 and 50% of the procuticle (Andersen, 1979), although the relative amount of chitin in cuticle can be variable (Andersen, 1979). For example, chitin has been shown to make up as much as ~60% of total cuticular mass in larval *Sarcophaga* (Fraenkel and Rudall, 1947); chitin content ranged from 24 to 41% of organic cuticular dry weight for adult Coleoptera (six species; Kramer et al., 1995); and chitin made up 15 to 20% of total cuticular dry weight in adult *Manduca* (7–12% of cuticular wet weight; (Kramer et al., 1995)).

Many materials are intimately associated with the cuticle, and it is difficult to separate those materials that are part of the cuticle from those that are simply attached. Because of the difficulty in effectively removing attached tissues and organs from the cuticle, we used chemical digestion (via sodium hydroxide) to isolate the cuticle and, thus, assess exoskeletal investment. This process preserves chitin (Shepherd et al., 1997; Steyskal et al., 1986; Zill et al., 2000; Ford and Stokes, 2006), and thus, chitinous procuticle makes up the bulk of what we measured in this study. It is important to note, however, that this approach did not allow us to quantify the protein component of the procuticle. We believe that because chitin accounts for a major fraction of cuticle and is crucial to the cuticle's structural integrity, chitin mass is currently the best, most practical measure of exoskeletal investment.

Material and Structural Properties of Insect Cuticle: Functional Tradeoffs

The success of insects in terrestrial environments is argued to be largely attributable to their exoskeleton (Chapman, 1998). The exoskeleton plays several different functional roles in insects, and tradeoffs among these roles (to support, protect, enable movement, reduce water loss, and facilitate gas exchange) are expected to influence exoskeletal composition and structure in insects. The strength, hardness, and elasticity of the exoskeleton provide the structural basis for many of these functions and are affected by both its material properties and its physical structure.

Material properties of exoskeleton (e.g., tensile strength, breaking strain, elastic recovery, hardness, toughness, brittleness) vary both among and within insect taxa, and the cuticle can be hard and stiff or soft and pliant (Fraenkel and Rudall, 1940; Fraenkel and Rudall, 1947; Hepburn and Chandler, 1976; Andersen et al., 1995). The tensile strength of the exoskeleton can, for example, range from high (elastic; high resistance to plastic deformation) in silkworm cuticle to low (brittle; low resistance to plastic deformation) in the cuticle of beetles and butterflies (Andersen, 1979). Cuticular elasticity can also differ among body parts within a single species, as has been observed in the differing elasticity of the locust hind tibia, forewing and pleural cuticle (Jensen and Weisfogh, 1962). Cuticular stiffness may also vary across developmental stages as has been found in *Drosophila* among larvae, pupae, and adults (Kohane et al., 2003). These differences in material properties are attributable to factors such as sclerotization, the relative amounts of chitin and protein, and the types of matrix proteins making up the cuticle (Hillerton and Vincent, 1979; Kramer et al., 1995; Andersen et al., 1996). Generally, the variation in material properties such as stiffness and flexibility of the exoskeleton probably reflects tradeoffs between different exoskeletal functions (e.g., protection vs. locomotion; Hepburn and Chandler, 1976).

Physical traits such as skeletal design (endoskeleton vs. exoskeleton), material thickness, and diameter also affect the strength of skeletal structures. For a given mass, exoskeletons have mechanical advantages over endoskeletons. Locke (1964) illustrated this difference using two appendages of equal diameter, where one was a solid central skeletal rod and one was a hollow skeletal tube which encased the appendage. Locke (1964) found that the hollow shell was three times stronger than a solid rod of the same mass and material. These differences in strength were observed because the strength and rigidity of cylindrical tubes increase as their external diameter increases (Price, 1997). For a given tube length and quantity of material, a hollow tube has a greater external radius than a solid rod. However, the strength advantage of exoskeleton may be lost at large body sizes when the hollow tube is constrained to a fixed quantity and type of material. Under these conditions, the overall strength of a hollow tube is compromised at large diameters because of decreased wall thickness and the increased possibility of buckling and collapse (Price, 1997). The only way to counteract this is to increase wall thickness by using more material. Without an increase in cuticular thickness, the strength of a shell type of skeleton would decline. There is thus a potential upper limit to body size for an animal possessing an exoskeletal system.

The structural characteristics of the exoskeleton, like its material properties, can vary within and among insect taxa. For example, the thickness of cuticle varies with species and life stage (Brown, 1975; Hartung et al., 2004). The increased wall thickness provides increased strength, and this type of investment in locomotor appendages potentially increases load carrying ability (i.e., the capacity to carry an increased total body mass). Increasing strength via increased tube thickness may simultaneously have material and/or metabolic costs that negate the benefits of increased strength.

Expected Variation in Exoskeletal Chitin Scaling in Arthropods

Overall, we predict that variation in the scaling relationship will manifest itself primarily as differences in the normalization constant of the scaling relationship (intercept), but that the slope of the relationship (exponent) will be relatively consistent across different locomotor and taxonomic groups. However, because of the many potential tradeoffs that may affect investment in the exoskeleton, we expect some variation in exoskeletal chitin scaling among different insect groups and lifestyles.

We predict that flying insects will invest less in exoskeletal chitin relative to body size than nonflying insects. Volant animals need to minimize nonessential weight because costs of transport are high (Tucker, 1970). Because exoskeletal mass is a significant contributor to total body mass, we expect flying insects to have relatively light exoskeletons. In addition, we predict that exoskeletal chitin scaling will exhibit a phylogenetic signal because closely related insects are more likely to be morphologically and physiologically similar to each other. Closely related species often also share life history traits that may lead to similarities in their relationship between exoskeletal chitin and body mass.

MATERIALS AND METHODS

Procedure

Adult insects were obtained by collection in the field (by net, by hand, and using pit-fall traps) and stored at −15°C. Animals were identified at the Museum of Southwest Biology (University of New Mexico, Albuquerque, New Mexico). Location, date, phylogenetic determination, and when possible, sex, were recorded for each individual. This sample included 551 individuals, distributed in 245 species, 91 families and 15 orders. Dry body mass of individual insects ranged from 0.1 to 2.41 g.

On collection, animals were euthanized by freezing, stored at −15°C, and removed only for purposes of identification until they could be weighed and dried. Masses were determined on a self-calibrating Mettler AX205 balance (readability ±0.00001g) immediately after removal from the freezer (recorded as "wet mass"). Animals were then dried at 60°C in an oven (VWR Scientific Mechanical Convection Oven, Model 1390FM, VWR, Cleveland, OH) and weighed at intervals until there was no change in mass (recorded as "dry mass"). Animals were then placed in glass vials containing petroleum ether (ether volume = ≥3× animal volume) for one week to extract ether-soluble lipids. After lipid extraction, animals were oven-dried and placed in glass vials containing a solution of 2 $mol \cdot l^{-1}$ sodium hydroxide, which dissolved tissues that were not exoskeletal chitin from the procuticle. Sodium hydroxide effects the removal of soft tissues of arthropods, and it is often used in invertebrate preparations to clear body contents while simultaneously preserving the chitinous exoskeleton (Steyskal et al., 1986; Shepherd et al., 1997; Zill et al., 2000; Ford and Stokes, 2006). Each sample went through two 4–6 h sodium hydroxide digestions (NaOH volume = ≥3× animal volume). These treatment times gave consistent results in preliminary trials (using *Eleodes*; n = 10). Large animals and individuals in which not all tissue was digested from the cuticle were subjected to a third digestion until their submergence in sodium hydroxide no longer yielded fluid discoloration or tissue degradation. Animals were then rinsed with distilled water three or more times and then dried to a constant mass in an oven at 60°C. Thorough rinsing is a critical step because residual NaOH will produce a precipitate that can adversely affect the mass measurements.

Statistical Analysis

There are several recognized methods for analyzing allometric data. We used both ordinary least squares (OLS) and standardized major axis (SMA) regression analysis (RMA V1.17, Bohonak and Van der Linde, 2004; SigmaPlot, SPSS Inc. 2001; SMATR V2.0, Falster et al., 2006) on species means, family means, and order means of chitin mass and dry mass. We also conducted independent contrasts on chitin mass and dry mass in Mesquite (Maddison and Maddison, 2006) using the PDAP module (MESQUITE V1.12) to make phylogenetic corrections. The topology of the phylogenetic tree was constructed at the family level using the method described by Grimaldi and Engel (2005), branch lengths of the tree were normalized, and OLS and SMA line-fits for log chitin mass vs. log dry mass were con-

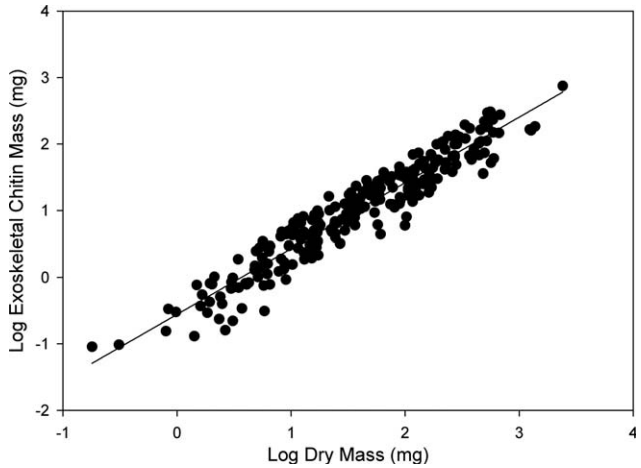

Fig. 1. The relationship between exoskeletal chitin mass and dry body mass. Each data point represents the average adult exoskeletal chitin mass and the average adult dry mass for an insect species. The slope of the relationship between chitin mass (mg) and dry body mass (mg) for insects varied slightly with method of statistical analysis, and it is represented by the equations: $M_{chitin} = a \times M_{dry}^{0.99}$ (OLS on species data), $M_{chitin} = a \times M_{dry}^{1.03}$ (SMA on species data; shown here), $M_{chitin} = a \times M_{dry}^{1.04}$ (OLS on independent contrasts), and $M_{chitin} = a \times M_{dry}^{1.08}$ (SMA on independent contrasts).

structed using normalized branch lengths and branch lengths set equal to one. The slopes of the scaling equations were only slightly different when branch lengths were varied (difference of 0.03), indicating that the data were not particularly sensitive to phylogenetic adjustment. Finally, we tested whether slopes for the relationship between means of chitin mass and dry mass were homogenous or heterogenous between insect orders using a General Linear Model (GLM; MiniTab 15.1.30.0 (MiniTab Inc. 2007)).

RESULTS

In general, chitin mass increased isometrically with body mass in terrestrial insects. There was no consistent effect of sex on chitin mass across phylogenetic groups ($P = 0.74$; paired t-test), so data from the two sexes were pooled. There was a consistent relationship between wet mass and dry mass across phylogenetic groups ($M_{dry} = -0.24 \times M_{wet}^{0.93}$ ($r^2 = 0.98$)), and because there was higher variance in wet mass than dry mass in our data (possibly partially attributable to differential freezing time and natural variance in animal hydration states), dry masses were used for allometric analyses.

The relationship between adult chitin mass and adult dry mass when analyzed as species averages for all insect species included in this dataset is isometric (species n = 245; Fig. 1). This was supported through both SMA regression and OLS regression; ($M_{chitin} = -0.632 \times M_{dry}^{1.033}$ (95% confidence interval [CI] = 0.995–1.073) for SMA analysis; $M_{chitin} = -0.558 \times M_{dry}^{0.987}$ (95% CI = 0.948–1.026) for OLS analysis). Neither of these slopes was statistically different from b = 1 (SMA $P = 0.09$; OLS $P = 0.51$), and both *were* statistically different from b = 1.1 (SMA $P = 0.0011$; OLS $P < 0.001$; b = 1.1 used to test for differences from the endoskeletal scaling exponent). In addition, the relationship between chitin mass and dry mass based on mean values for species was not statistically different from the relationship between chitin mass and dry mass based on individual values, or mean values for families or orders (data not shown). None of these exponents were significantly different from 1 ($P < 0.05$). All figures shown in this article are based on regression analysis of species averages.

Chitin scaling equations from independent contrast analysis were marginally different from the results obtained without phylogenetic adjustment. Specifically, the chitin scaling exponents derived from independent contrast analysis were b = 1.04 (OLS for intact branches), b = 1.01 (OLS for branch lengths equal to 1), b = 1.08 (SMA for intact branches), and b = 1.05 (SMA for branch lengths equal to 1).

Different taxonomic groups within Insecta showed differences in chitin investment. For example, chitin of odonates (dragonflies) and coleopterans (beetles) averaged 16 and 37% of dry mass, respectively. Using species averages for these two orders, which each exhibited several orders of magnitude variation in body mass, yielded scaling exponents that were statistically different from each other (0.99 and 1.09, respectively; Fig. 2; $P < 0.0001$; GLM with Order as model, chitin mass as response, and dry mass as covariate). To further explore taxon-specific chitin scaling relationships, allometric equations were determined using SMA

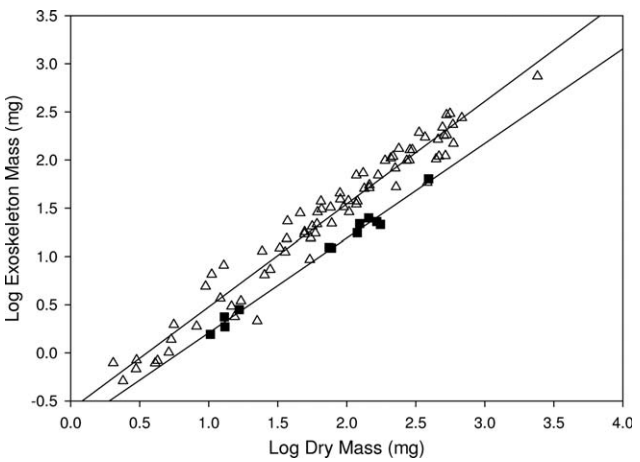

Fig. 2. The exoskeletal chitin scaling relationship for Coleoptera and Odonata. Each data point represents average adult exoskeletal chitin mass and the dry mass for a species. The hollow triangles represent species of Coleoptera, and the solid squares represent species of Odonata. Slopes (SMA; 1.09 and 0.99) are significantly different from one another ($P < 0.0001$).

TABLE 1. Allometric scaling of exoskeletal chitin mass with dry body mass in insects

	No. species	Slope	%Chitin, 10 mg insect	%Chitin, 50 mg insect	r^2	Orders of magnitude	95% CI of slope	P (b = 1)
Class Insecta	245	1.033	23.3	25.0	0.913	4	0.995–1.073	0.09
Insect Order								
Blattodea	4	—	—	—	—	3	—	—
Coleoptera	77	1.091	23.2	28.2	0.955	4	1.038–1.145	0.001
Diptera	21	1.025	20.7	21.9	0.793	3	0.830–1.267	0.808
Hemiptera	13	0.979	24.5	23.4	0.854	4	0.769–1.245	0.848
Hymenoptera	54	1.021	41.1	43.0	0.945	4	0.956–1.091	0.520
Isoptera	1	—	—	—	—	n.a.	—	—
Lepidoptera	19	0.937	27.4	24.1	0.935	4	0.826–1.064	0.295
Mantodea	3	—	—	—	—	3	—	—
Megaloptera	2	—	—	—	—	n.a.	—	—
Neuroptera	1	—	—	—	—	n.a.	—	—
Odonata	12	0.987	16.5	16.0	0.990	3	0.924–1.054	0.668
Orthoptera	34	1.109	8.9	11.2	0.823	3	0.958–1.283	0.163
Phasmatodea	2	—	—	—	—	n.a.	—	—
Raphidioptera	1	—	—	—	—	n.a.	—	—
Zygentoma	1	—	—	—	—	n.a.	—	—

Comparison of the allometric scaling relationships for exoskeletal chitin between insect orders. Slopes presented here were derived via standardized major axis regression analysis of species averages.

and OLS regression analysis for all insect orders with ≥10 species represented in our dataset, and Ancova analysis was again performed to determine if slopes were statistically different from one another. Regression analysis conducted via SMA generally produced slopes that were slightly higher than those generated using OLS analysis (e.g., Hymenoptera SMA b = 1.02 *vs*. OLS b = 0.99, Hemiptera SMA b = 0.98 *vs*. OLS b = 0.91, Coleoptera SMA b = 1.09 *vs*. OLS b = 1.06, Diptera SMA b = 1.03 *vs*. OLS b = 0.92, and Orthoptera SMA b = 1.11 *vs*. OLS b = 1.01), and SMA statistical results are presented in this article for comparison of orders (Table 1). Although analysis of chitin scaling *across* Class Insecta yielded a slope of 1.03, SMA slopes for individual insect orders ranged from 0.94 (Lepidoptera) to 1.11 (Orthoptera; Table 1 and Fig. 2). The slopes for all orders excluding that of Coleoptera were statistically indistinguishable from b = 1 (Table 1), and comparison between insect orders demonstrated that chitin scaling slopes were statistically heterogenous ($P < 0.0001$; GLM analysis with Order as model, chitin mass as response, and dry mass as covariate) when all groups were included in the analysis, but statistically homogenous ($P > 0.05$) when Coleoptera and Hymenoptera were excluded. In sum, the isometric chitin body mass relationship for Class Insecta seems to (1) have a phylogenetic signal and (2) also reflect isometry at the taxonomic level of insect order, for all groups except Coleoptera.

The chitin scaling relationship also varied with locomotor group. Flying insects (129 species from Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Megaloptera, Neuroptera, Odonata, and Orthoptera that have wings and are known to fly) and nonflying insects (74 species from Coleoptera, Hemiptera, Hymenoptera, and Orthoptera that do not have functional wings) tended to have quasi-isometric slopes for the scaling of chitin with body mass ($P > 0.05$ for b = 1 for flyers and nonflyers using SMA; although $P < 0.01$ for flyers and $P > 0.05$ for nonflyers using OLS; Fig. 3). Flying insects exhibited a slightly lower slope than nonflying insects ($M_{\text{flying insect chitin}} = -0.56\ M_{\text{dry}}^{0.97}$ (95%CI = 0.918–1.024) for SMA; $M_{\text{flying insect chitin}} = -0.48\ M_{\text{dry}}^{0.92}$ (95%CI = 0.868–0.974) for OLS, vs. $M_{\text{non-flying insect chitin}} = -0.55\ M_{\text{dry}}^{1.03}$ (95%CI = 0.972–1.095) for SMA; $M_{\text{non-flying insect chitin}} = -0.49\ M_{\text{dry}}^{1.00}$ (95%CI = 0.937–1.060) for OLS), and these slopes differed from one another statistically ($P < 0.0001$; GLM analysis with locomotion as model, chitin mass as response, and dry mass as covariate). Flying insects, however, tend to have proportionally less chitin than nonflying insects for the majority of observed insect masses. For example, for a "typical" 100 mg animal (dry mass), chitin mass is 25 mg for a flying insect and 31 mg for a nonflying insect. However, these lines cross. Our data indicate that a "typical" 10 mg flying insect would have approximately the same chitin mass as a "typical" 10 mg nonflying insect (~28% of dry body mass), and at very low masses, flying insects may have proportionally more chitin than nonflying insects.

DISCUSSION

Scaling of Exoskeletal Chitin in Insects

General patterns. Although our dataset does not encompass the entire range of ecological and morphological possibilities for insects, our results show that insects (except perhaps beetles) devote essentially the same fraction of their body mass to exoskeletal chitin regardless of body size. Our results also indicate that size independent investment in exoskeletal chitin, as indicated by normal-

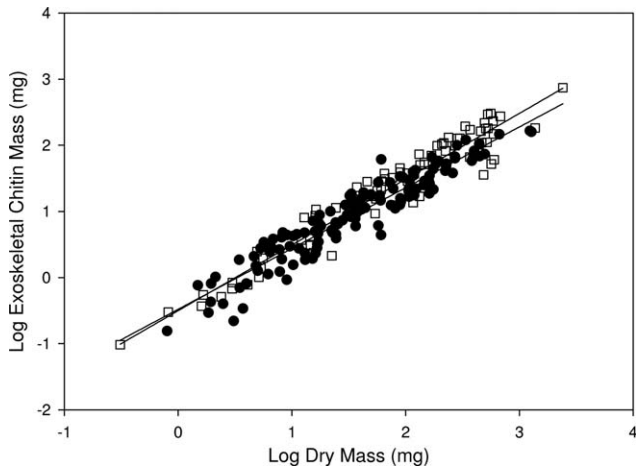

Fig. 3. The exoskeletal chitin scaling relationship for flying vs. nonflying insects. Each data point represents the average adult exoskeletal chitin mass and the dry mass for a species. The hollow triangles represent species that fly, and the solid squares represent species that do not fly. Slopes (SMA; 0.97 and 1.03) are significantly different from one another ($P < 0.0001$).

ization constants, varies with locomotor mode and taxonomic group. Our observations of isometry in insect exoskeleton differs statistically from the positive allometric relationship found for the endoskeletons of vertebrates ($P \leq 0.001$ for $b = 1.1$), and for most other physical structures (e.g., plants, buildings; McMahon and Bonner, 1983; Peters, 1983; Calder, 1984). Our observations also suggest that the earlier conclusion of positive allometry for the scaling of arthropod exoskeleton based only on arachnids (Anderson et al., 1979) was premature. Finally, our results have implications for understanding the limits on body size in insects, and for the dual role of exoskeleton as both a structural support system and an integumentary system.

Isometry of exoskeletal chitin. Insect exoskeletal chitin could hypothetically scale with respect to body mass in three different ways. Exoskeletal chitin could comprise a constant proportion of body mass ($b = 1$), which is typical of hearts, lungs, and muscles in vertebrates, and it is probably based on the physiological interdependence of organs placing top-down constraints on organ size (Calder, 1984) and/or the geometric scaling-up of organs based on basic structural principles (Prothero, 1996). Exoskeletal chitin could make up a relatively larger proportion of body mass in larger animals ($b > 1$), which is typical of vertebrate endoskeleton (e.g. Prange et al., 1979), in order to maintain the strength needed to support the animal's weight. Or, exoskeletal chitin could make up a relatively smaller proportion of body mass in larger animals ($b < 1$), which is typical of integument (Pace et al., 1979), because the exoskeleton covers the surface of an animal which scales as $M^{2/3}$.

We found an isometric relationship between exoskeletal chitin mass and dry body mass ($b = 1$). Thus, the support requirements in insects seem to be satisfied by increasing exoskeletal chitin mass in direct proportion to body mass. These data are supported by the observation that leg diameter does not seem to increase, relative to length, as body size increases in arthropods (Prange, 1977). Increased strength with isometric scaling is probably partially achieved by increases in integumental thickness with increasing body size as is commonly seen in vertebrates (Calder, 1984). Evans and Sanson (2005) showed that there is a significant increase in maximum cuticle thickness with increased body size in beetles and moths ($P < 0.05$ for both), although maximum cuticle thickness was not a good predictor of animal size ($b = 0.002$ and $b = 0.004$, respectively). Data for grasshoppers indicate that exoskeletal thickness also increases in the legs of larger and older insects (2nd instar vs. adult grasshoppers; Hartung et al., 2004). It is unclear, however, whether whole body exoskeleton also gets thicker. Changes in the relative amounts of protein and chitin, the combinations of matrix proteins, and the architecture of the chitin filament also alter the physical properties of arthropod cuticle (Hillerton and Vincent, 1979; Andersen et al., 1996), and may contribute to isometric scaling by increasing cuticular strength without an increase in the relative investment in cuticle. The current analysis precludes a determination of which compositional changes are occurring and their direct effects on cuticular strength.

Variation in exoskeletal chitin. We found variation in exoskeletal chitin scaling between some taxonomic groups, and with respect to locomotor mode, independent of body mass. Flying insects had proportionally less exoskeletal chitin relative to body mass than nonflying insects, and they increased their exoskeletal chitin investment at a lower rate as mass increased. Different taxonomic groups of insects also exhibited varying degrees of investment in exoskeletal chitin (e.g., Coleoptera intercept > Odonata intercept; Fig. 2. Taxonomic differences in exoskeletal chitin allometry are likely due to tradeoffs in exoskeletal function. For example, dragonflies may minimize skeletal investment for increased agility and lowered metabolic expenditure during flight, because the minimum cost of transport scales negatively with mass (Taylor et al., 1970; Lighton, 1985) and the cost of transport is inversely proportional to speed (Taylor et al., 1970). Beetles, in contrast, typically invest in a robust and relatively heavy exoskeleton, which probably offers them more support and protection (Evans and Sanson, 2005). Taxonomic differences in sclerotization (Andersen et al., 1996) and cuticle composition (e.g., chitin:protein ratios; Kramer et al., 1995) and/or insect shape

undoubtedly act synergistically with the observed differences in exoskeletal chitin investment to produce differences in exoskeletal function. We did not observe a consistent effect of sex on exoskeletal chitin investment across phylogenetic groups, although we would still expect one to exist within groups that exhibit sexual dimorphism (because ornaments tend to increase disproportionately with body size; Kodric-Brown et al., 2006). The data presented here are probably insufficient to address sex differences in support structures. Finally, we acknowledge that by focusing on the variation in exoskeletal chitin investment in only *terrestrial* insects, this dataset is limited in its ability to generalize patterns across all insects and to assess the role of gravitational support in structuring exoskeletal allometry. A comparison of exoskeletal investment between aquatic and terrestrial insects would correct this deficiency, and we are currently evaluating exoskeletal investment of marine and freshwater arthropods.

Deviation of data from literature values for exoskeletal scaling. The study by Anderson et al. (1979) indicated that the external skeletons of spiders, freshwater mollusks, and bird eggs scale similarly to endoskeletons of birds, land mammals, and whales (Smith and Pace, 1971; Prange et al., 1979), and that, if anything, investment in supportive tissue increases at an even *faster* rate as body size increases in organisms with exoskeletons than it does in organisms with endoskeletons. The data of Anderson et al. (1979), while interesting, are hardly representative of arthropods in general because only three species of spiders (n = 61; body size range of 25 mg to 1.2 g) were represented, data of individuals were plotted, and individuals included were a combination of immature and adult animals. The study by Wheatley and Ayers (1995) possesses similar limitations. Wheatley and Ayers (1995) suggest that the scaling of calcium and inorganic contents can be used as an index of supportive tissue investment in crustaceans, and they argue that intraspecific intermolt scaling of cuticular minerals (b = 1.3) agrees with the published values for positive allometric scaling of exoskeletons (Anderson et al., 1979). However only one species of crustacean was represented in their study (n = 45; body size range of 0.012–33 g), and their general mineral scaling exponents ranged from 0.93 to 1.27. Our data represent 245 species of adult insects (n = 551; body size range of 0.2–6.13 g), and although restricted to insects, provides a much broader picture of the arthropod scaling exponent than was possible for Anderson et al. (1979) or Wheatley and Ayers (1995). We conclude that insect exoskeletal chitin generally scales isometrically and that this pattern is robust for our sample; the exponent is 1 regardless of whether the data are plotted and analyzed as individuals, species averages, family averages, or order averages.

Deviation of data from literature values for endoskeletal scaling. In animals with endoskeletons, skeletal investment and strength increases disproportionately with body mass to provide biomechanical support (Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984). This is because if skeletal materials and density are constant, strength is proportional to mass to the two thirds power ($S^{L/2} \alpha M^{L/3}$; or $S \alpha M^{2/3}$). This means that for animals with similar body plans, the safety factors for load bearing and for withstanding the forces of locomotion would be compromised at larger body masses. To overcome these mechanical constraints, animals must scale the skeleton with positive allometry, evolve weight-specific force reduction (that matches mass-specific decreases in tissue cross-sectional area), or restrict their size range (Biewener, 2000).

The scaling of structural support has been studied in diverse vertebrate taxa (Mitchell et al., 1945; Prange and Christman, 1976; Prange et al., 1979; Miller and Birchard, 2005), and in most cases, the endoskeleton scales with positive allometry. This means that as body size increases, endoskeletal mass makes up a relatively larger proportion of total body mass. This holds true for most terrestrial vertebrates (birds = 1.07, land mammals = 1.09, rattlesnakes = 1.17, turtles = 1.2 (Prange and Christman, 1976; Prange et al., 1979; Miller and Birchard, 2005), but not for teleost fish and amphibians (fish = 1.03 (Reynolds and Karlotski, 1977), amphibians = 1.0004 (Leclair et al., 1993)). The endoskeletons of vertebrates do not, however, scale to 1.33 of body mass ($M^{4/3}$), as would be predicted if they were designed only to resist compression. Instead, the supportive tissue of land vertebrates scales with body mass with exponents between 1.1 and 1.2. The universal tendency of endoskeleton to scale with positive allometry, but less than as $M^{1.33}$, is likely due to a trade off between the different functions of the skeleton; whereas increased skeletal investment offers more support, decreased skeletal investment means there is less total weight to be supported and transported.

Overall, the evidence for a positive allometric relationship between endoskeleton and body mass is well supported, both theoretically and biologically. However, the isometric relationship between insect exoskeletal chitin mass and body mass that we document in this study is also robust. If exoskeletal chitin is indeed a good index of exoskeletal investment, insects are clearly doing something different than vertebrates.

Exoskeleton's role in body size limitation. Most hypotheses that try to explain body size limitation in insects are based on one of two mechanisms that are believed to be compromised as body

size increases: oxygen delivery (Graham et al., 1995; Dudley, 1998; Kaiser et al., 2007) and structural support (Kennedy, 1927; Schmidt-Nielsen, 1975; Price, 1997). For any group of animals that exhibit positive allometry for support structures, there must theoretically be a size limit beyond which an increase in size is either not possible or not extremely costly to maintain. Based on Anderson et al. (1979)'s data, arthropods should possess such a structural support–based size limit. In addition, if the maximum size for both vertebrates and arthropods were indeed constrained because of increasing allocation to structural support as body size increases, it would be possible for the scaling exponent for exoskeletal chitin in insects to be greater than the scaling exponent for endoskeleton in vertebrates, because the largest known arthropod is much smaller than the largest known vertebrate. Interestingly, we instead found that the exoskeletal chitin exponent is smaller than the endoskeletal exponent, and that exoskeletal chitin investment does not increase as an animal gets larger. The isometric pattern that we document here thus suggests that exoskeleton may not limit insect body size. Can our results be extrapolated to all arthropods? A small interspecific dataset for marine crustaceans, some of which have body sizes two orders of magnitude larger than extant insects, suggests that exoskeletal isometry may indeed hold true for arthropod groups other than insects (Lease, unpublished data). However, data of Wheatley and Ayers (1995) and Anderson et al. (1979) certainly suggest that more data are needed to ascertain if this is true.

A developmental feature of insects that may influence maximum insect body size is the fact that support is temporarily lost during and directly after ecdysis (molt), but before sclerotization (hardening) (Andersen et al., 1996; Chapman, 1998). At this soft stage, bodies of small insects maintain their form by hydrostatic pressure (because the effect of gravity is relatively small), but the structural and functional integrity for larger insects might be compromised (Berenbaum, 1996; Price, 1997; Calabi, 1998). Thus, it is possible that selective pressure on body size occurs during the temporary loss of structural support during ecdysis.

Integument + endoskeleton = exoskeleton? Insects were shown in this study to have isometric scaling of exoskeletal chitin mass, which failed to support the hypothesis that exoskeletal investment scales with body surface area. Surface-area-to-volume relationships for simple shapes such as cylinders demonstrate that as body size increases, relative surface area decreases. If exoskeleton scaled primarily with respect to the surface area that it covers, it should scale as $\sim M^{2/3}$. However, because integument thickness must increase to maintain yield and tensile strength as body size increases, we would expect integument mass to scale with an exponent >2/3. The scaling of the integument in vertebrates indeed exhibits slopes of 0.90–0.94 (Pace et al., 1979; Lindstedt and Calder, 1981; Calder, 1984), which has been explained as being due to a combination of a decrease in relative surface area, an increase in absolute surface area, and an increase in integumental thickness as animals increase in size (Calder, 1984).

The scaling exponent of vertebrate integument is, therefore, close to 0.9. For mammals, this means that the combination of (decreasing; 0.9) skin mass and (increasing; 1.1) skeletal mass accounts for a constant percentage of body mass, regardless of body size (Lindstedt and Calder, 1981; Calder, 1984). This is interesting when considering that in insects, the exoskeleton is simultaneously functioning as a support structure and an integument, and it scales isometrically. For mammals, these functional roles are held by different body systems, but, when they are combined, their fractional contribution to body mass is the same as in insects.

Potential Issues Affecting Data Interpretation

Our data indicate that the exoskeletal chitin scaling relationship with dry body mass across insects is isometric or very close to it. We found that this relationship between chitin mass and dry mass was consistent regardless of whether our data were analyzed as individuals, species means, family means, or order means. However, SMA regression analysis on phylogenetically corrected values for exoskeletal chitin yields an allometric slope that is slightly higher than 1 ($b = 1.08$). Although confidence intervals generated during this phylogenetically corrected regression analysis overlap $b = 1$, this leaves open the possibility that there might be a slight positive allometry. This issue can probably only be resolved by increased sampling effort.

Our study also assumes that the protein content of the cuticle is a constant fraction of exoskeletal mass. The limited literature available suggests that the protein to chitin ratio of the cuticle may be highly variable across and within insects (Fraenkel and Rudall, 1940; Hackman and Goldberg, 1958) and as a consequence this variation could affect our scaling relationship. This phenomenon has not been evaluated systematically and is beyond the scope of the present study. We acknowledge that if the protein component of cuticle scales with positive allometry, it could indicate that larger insects require greater structural investment, and that total exoskeletal mass exhibits positive allometry rather than isometry. To

address this issue in part, one could measure the protein content of exoskeleton across a range of body sizes of a single body location for a single group of insects.

CONCLUSIONS

Our results indicate that exoskeletal chitin scales isometrically with dry body mass across many insect groups and across four orders of magnitude of body size. Thus, exoskeletal chitin comprises a constant proportion of insect body mass, regardless of body size, for most orders of insects. However, our results also indicate different normalization constants for exoskeletal chitin, across different taxa. The high degree of physiological and ecological variation within Class Insecta probably accounts for most of the allometric variation observed in this study, and indeed, we found that both phylogeny and locomotor mode affected investment in exoskeletal chitin. We are continuing to explore deviations in exoskeletal investment with respect to sex, and aquatic vs. terrestrial lifestyle. Despite the observed allometric variation, the isometric scaling of exoskeleton chitin documented in this study leads us to conclude that (1) the scaling of insect exoskeletal mass with body size may deviate from what was previously described for arthropods, (2) exoskeletal chitin mass of insects scales differently with body size than endoskeletal mass of vertebrates, and (3) investment in exoskeleton may not be the primary limit on body size in insects, because exoskeletal chitin investment increases in direct proportion to body size.

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