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Morphometric condition as a measure of energetic content and reproductive potential in *Dactylotum variegatum*

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Abstract

Morphometric condition (*i.e.*, condition estimated from some aspect of body size) is frequently calculated from mass-size residuals, and is often used to infer something about an animal's energetic state. However, controversy exists about the validity of using mass-size residuals for this purpose. Recent work suggests the approach is appropriate in mammals, but it is unclear if morphometric condition is useful in the context of understanding the energetic state of grasshoppers and whether differences in morphometric condition are associated with variation in fecundity-related characteristics in females. To address this question, I examined male and female *Dactylotum variegatum*, sampled from a mosaic of grazed and ungrazed grasslands in southeastern Arizona, to determine whether morphometric condition corresponded with lipid and glycogen levels in males and a variety of reproductive characteristics in females. *D. variegatum* was found to be strongly sexually dimorphic relative to mass and femur length. The relationship between mass and femur length was linear for both males and females, an important assumption underlying the use of mass-size residuals as a measure of condition. Structurally larger males had more lipid and glycogen content. After controlling for the effect of size on lipid and glycogen content, a positive relationship was found between morphometric condition and lipid and glycogen levels, although the relationship was relatively weak for glycogen. In females, femur length, mass, and morphometric condition were not significantly associated with the number of ovarioles or the mean number of eggs or corpora lutea per ovariole. Only the mean number of resorption bodies per ovariole was significantly related to mass and morphometric condition, with larger and better conditioned females having fewer resorption bodies. The results of this study suggest that mass-size residuals in this species of grasshopper are related to energetic condition, but that other factors, such as variation in water and protein content, may weaken the relationship. Morphometric condition probably has limited value in estimating fitness of wild-caught *D. variegatum* females.

Key words

Arizona, *Dactylotum variegatum*, condition, corpora lutea, fecundity, fitness, glycogen, lipids, ovarioles, mass-size residuals, resorption bodies

Introduction

Some aspect of body size is often used by ecologists to estimate the "condition" of an animal. Although often vaguely defined, condition is typically used to refer to the energetic state of an animal, with animals in good condition having more energy reserves than animals in poor condition (Schulte-Hostedde *et al.* 2005). Condition can have important fitness consequences, and many studies have found a positive relationship between condition and reproductive potential in females (*e.g.*, Moya-Laraño 2002, Beauplet & Guinet 2007, Wauters *et al.* 2007). Using morphometric condition (*i.e.*, some aspect of body size) as a surrogate for energetic condition is attractive for several reasons. The study organism does not have to be destructively sampled and taking morphometric measurements is faster, easier, and less costly than quantifying energy reserves or reproductive potential.

In the past, two general methods have been commonly used to measure morphometric condition in animals. One way is to use a ratio between mass and some measure of structural body size (Schulte-Hostedde *et al.* 2001). Several studies have shown that this method is not ideal, because the ratios are not independent of body mass or size (Lidicker & Ostfeld 1991, Jakob *et al.* 1996). A more frequently employed method is the use of the residuals of a regression of body mass on body size (see Green 2001 for a review of this method in vertebrate studies); this method has been used in several studies of morphometric condition in grasshoppers (*e.g.*, Wall & Begon 1987, Martin-Alganza *et al.* 1997, Reinhardt 2001). Because grasshoppers have determinate growth, the size of the exoskeleton at adult emergence remains constant, and mass is subsequently added (Wall & Begon 1987). Thus, the regression represents the expected relationship between each individual's exoskeleton size if all individuals gain a typical amount of mass following emergence and the residuals serve as an index of adult condition (Wall & Begon 1987). Because males continue to gain mass after emergence, and females continue to gain mass after emergence up to their first oviposition, these residuals are believed to reflect the adult's ability to obtain and accumulate resources (Wall & Begon 1987).

In the last decade, the practice of using residuals has come under increased scrutiny and criticism (*e.g.*, Jakob *et al.* 1996, Kotiaho 1999, Green 2001, Hayes & Shonkwiler 2001), primarily because of the assertion that many of the assumptions underlying the use of the method are probably frequently violated. However, a recent paper by Schulte-Hostedde *et al.* (2005) suggests that using mass-size residuals as a measure of condition is often appropriate and that their use appears to satisfy the most important of these assumptions.

Unfortunately, few studies have evaluated how closely morphometric condition relates to energetic content, and most have focused on vertebrates (*e.g.*, Krebs & Singleton 1993, Virgl & Messier 1993, Schulte-Hostedde *et al.* 2001, Ardia 2005). I am unaware of research that has examined whether morphometric condition in grasshoppers reflects energetic condition, although some work has examined the relationship between morphometric condition and reproductive characteristics and fecundity (*e.g.*, Wall & Begon 1987, Martin-Alganza *et al.* 1997, Reinhardt 2001).

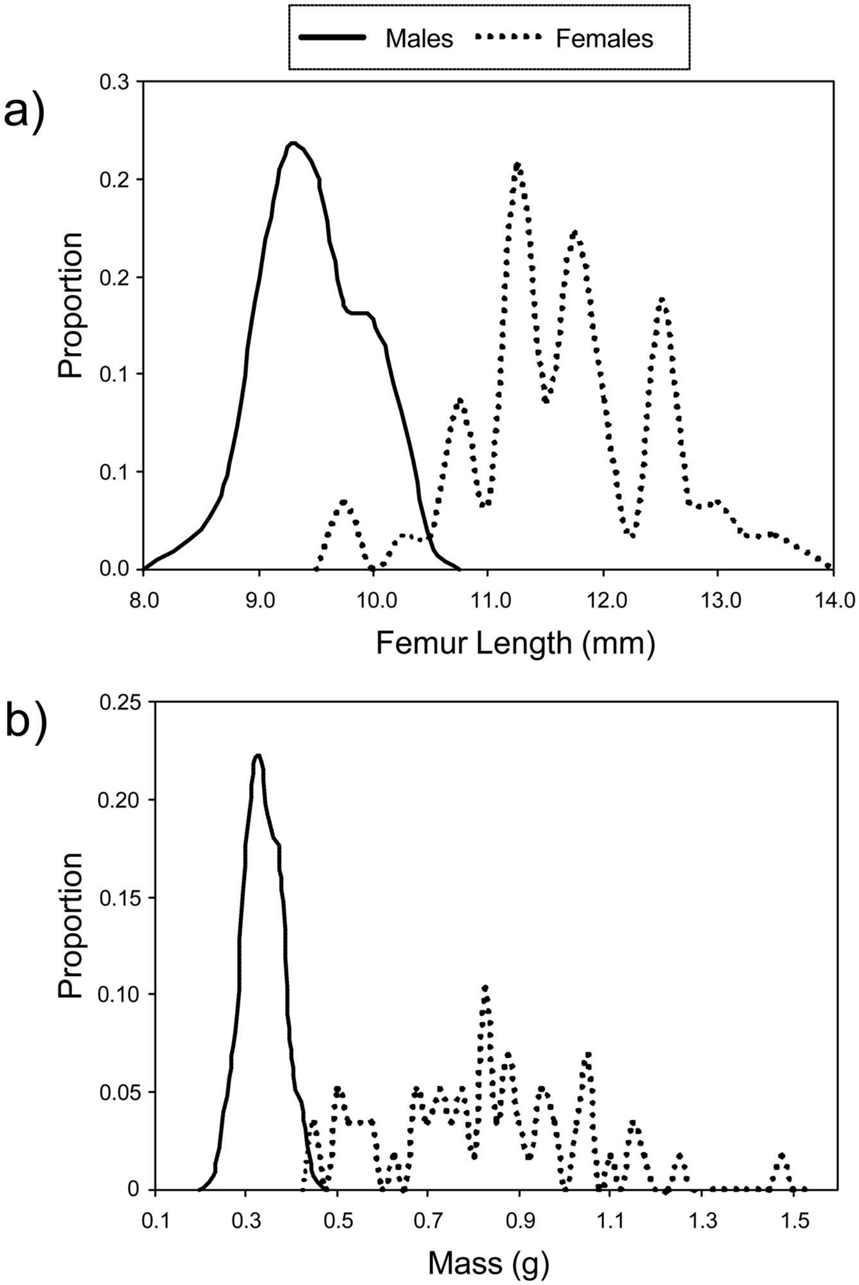

Fig. 1. Size distribution of males and females relative to **a)** femur length and **b)** mass.

To determine how representative morphometric condition (as measured by mass-size residuals) is of energy reserves, I examined *Dactylotum variegatum*, a common grasshopper of the southwestern United States. I collected individuals from a mosaic of grazed and ungrazed grasslands in southeastern Arizona. Here livestock grazing practices and the existence of a large, ungrazed, nature sanctuary, create a heterogeneous environment, where differences in grasshopper condition are expected to be large. In fact, variability in biotic and abiotic factors associated with livestock grazing in the area, affect insect communities (DeBano 2006a) and density of *D. variegatum*, in particular (DeBano 2006b). Grasshoppers living in this heterogeneous environment are expected to encounter a continuum of environmental conditions within the course of their lifespan. Thus, the general goal of the study is to determine whether variability in morphometric condition, regardless of whether this condition is associated with a particular grazing regime, is correlated with energy reserves in males and reproductive characteristics of females.

The specific objectives of the study were to: 1) document variability and sexual dimorphism in mass and body size of *D. variegatum* in southeastern Arizona; 2) examine the relationship between mass and femur length (not only for descriptive purposes but also to determine if assumptions for using mass-size residuals as a measure of morphometric condition are met); 3) determine if structurally larger males have more energy reserves in the form of glycogen and lipid content, simply by virtue of being larger; 4) determine if larger females have greater potential fecundity; and 5) determine whether there is a relationship between morphometric and physiological measures of condition in males and between morphometric condition and fecundity in females.

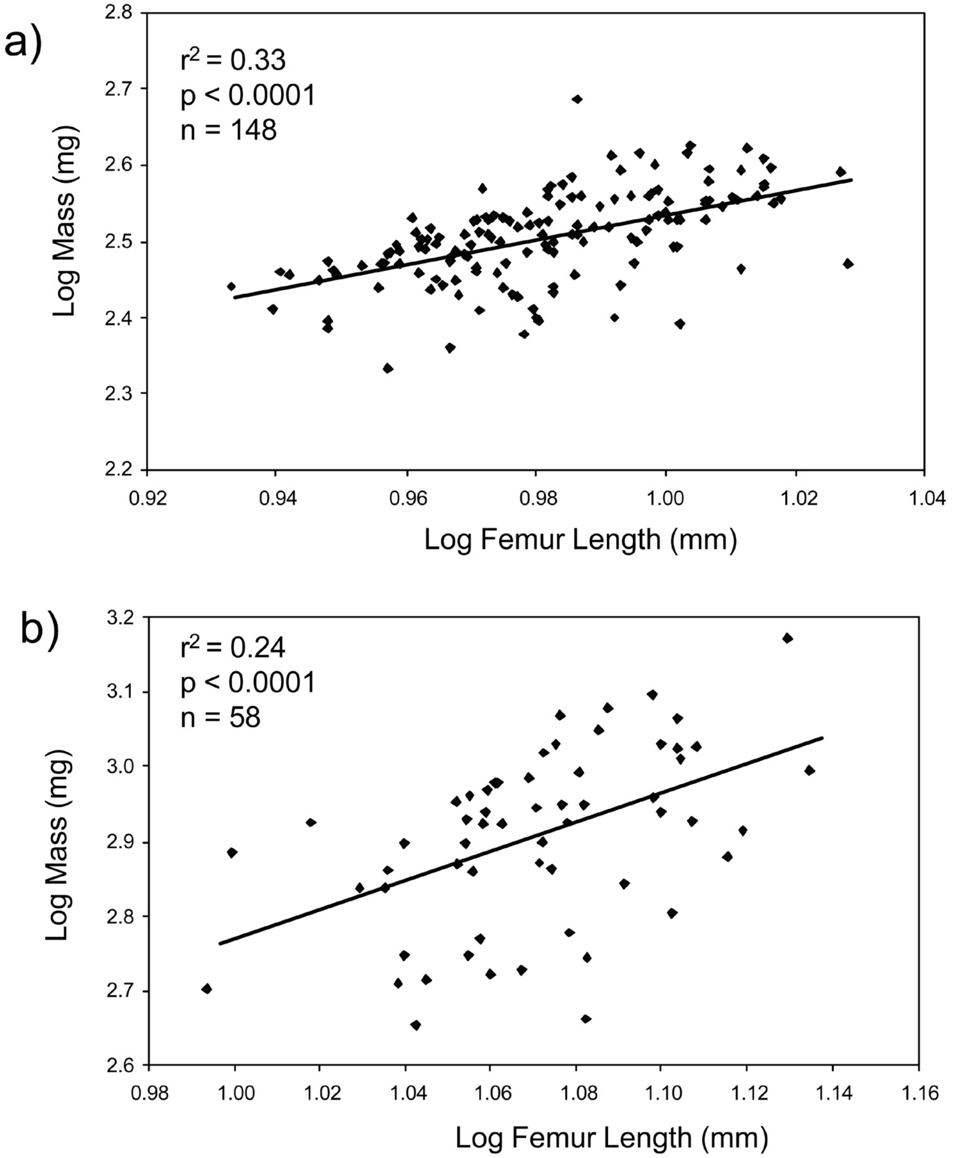

Fig. 2. Relationship between $\log_{10}$ mass (mg) and $\log_{10}$ hind femur length (mm) for **a)** males (y=1.732x-0.194) and **b)** females (y=1.995x+0.769).

Methods

Study Sites and Collection Methods.— Grasshoppers were collected at 10 sites in semi-arid grasslands of southeastern Arizona. Five of the sites were located at Audubon's Appleton-Whittel Research Ranch, a 3160-ha sanctuary ungrazed by cattle since 1968. The other five sites were located on grazed grasslands ranging from 1 to 20 km from the Research Ranch. Individuals were randomly collected from these sites on 10 days in 1995 (August 9, 18, 22, and 26; September 4, 12, 25, 26, and 27; October 11). Sites were sampled by walking random transects and collecting all individuals visible from those transects, or by using a ring collecting method as described by Onsager and Henry (1978). Sixty-four females and 148 males were collected and frozen in liquid nitrogen.

Size and Morphometric Condition in Males and Females.—I measured hind femur length and wet mass of all grasshoppers. For consistency, I removed legs and wing pads of grasshoppers before weighing because some individuals were missing limbs or wing pads. Femur length was measured using a Power Macintosh 8500 computer with video camera and a public-domain NIH image analysis package. Bodies were positioned in the same orientation relative to the camera and femur lengths were measured twice and averaged. Morphometric condition was estimated by the residuals of $\log_{10}$ mass regressed against $\log_{10}$ femur length.

Energetic Condition.— Energetic condition in males was estimated by measuring lipid and glycogen levels. Previous work has shown that lipid levels are a good estimate of long-term energy reserves and glycogen levels are a good estimate of short-term energy reserves (Gilbert & Schneiderman 1961, Beenakkers 1969, Van Handel 1974). After weighing, each grasshopper was homogenized. Lipids were extracted from one quarter of the homogenate with chloroform-methanol, using a method modified from Van Handel (1965); they were quantified by comparing spectrophotometric values to standards using a modified vanillin method (Holwerda *et al.* 1977). The pellet remaining after chloroform-methanol extraction contained the glycogen fraction, which was solubilized in ethanol and quan-

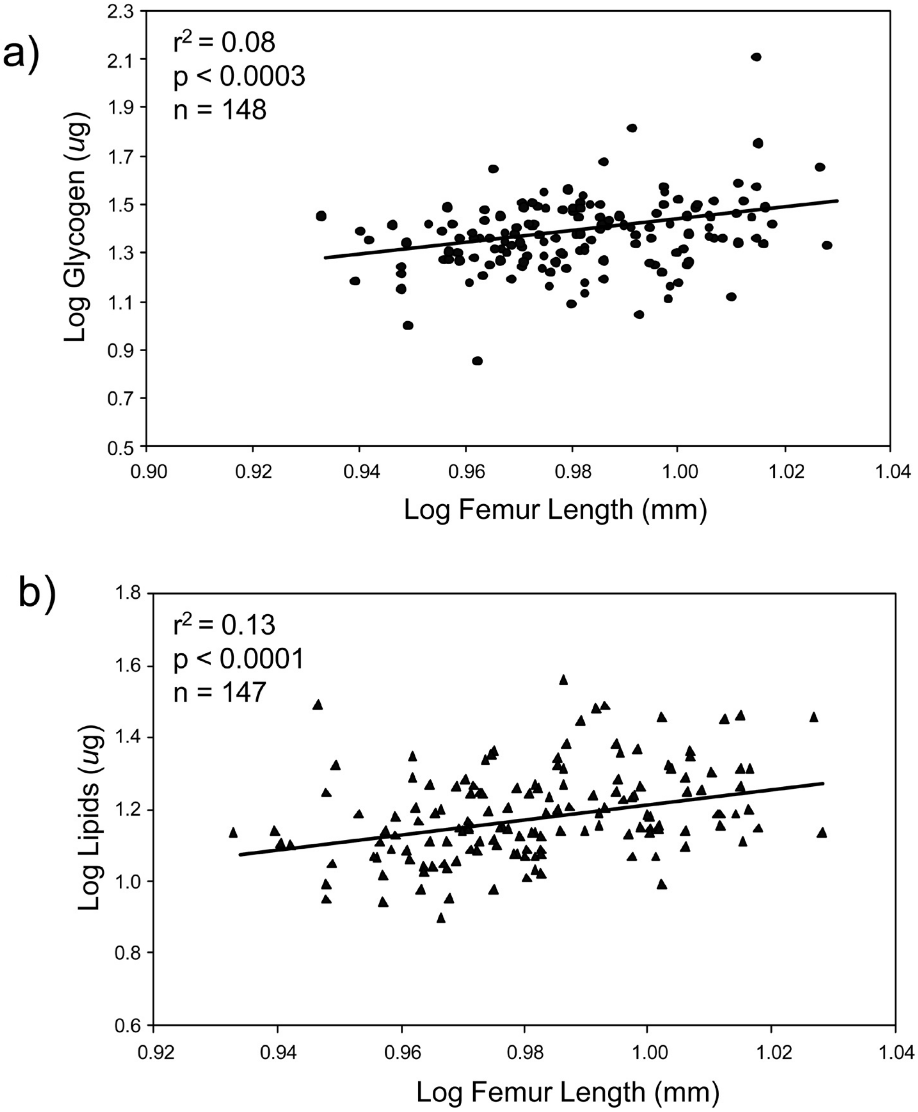

Fig. 3. Relationship between **a)** log$_{10}$ glycogen content (μg) and log$_{10}$ hind femur length (mm) [y=2.24x-0.832] and **b)** log$_{10}$ lipid content (μg) and log$_{10}$ hind femur length (mm) [y=2.296x-1.066] for males.

tified by comparing spectrophotometric values to standards using anthrone as the color reagent (Holwerda *et al.* 1977).

Total amounts of lipid and glycogen were first regressed against femur length to determine if larger individuals had more energy reserves. If so, then the amounts of lipid and glycogen were regressed against femur length and residuals were used in analyses comparing energetic reserves with morphometric condition. This resulted in measures of energetic condition that were controlled for differences simply due to body size.

Energy reserves in females were not quantified, because a large portion of their energy reserves are determined by number of eggs, the stage of development of those eggs, and how many egg pods have been laid (Weed Pfeiffer 1945, Cheu 1952, Uvarov 1966). In one study, up to one-third of the total amount of fat was lost after one oviposition (Weed Pfeiffer 1945). Therefore, the amount of energy reserves in females at any particular time may have little to do with the overall condition of the organism.

Female Fecundity.— Several variables related to fecundity were measured in females, including the number of ovarioles. Number of ovarioles estimates the maximum reproductive potential of grasshoppers because the number of eggs laid in a pod by a female is equal to or less than the number of ovarioles she possesses (Uvarov 1966, Dearn 1977, Stauffer & Whitman 1997). In addition, I also counted the number of eggs per ovariole and estimated the number of eggs laid and the number of eggs reabsorbed, by counting the number of corpora lutea and resorption bodies respectively. When grasshoppers lay an egg pod, a white body, the corpus luteum, is visible at the proximal end of the ovariole; when an egg is reabsorbed, a yellow body, the resorption body, is visible at the proximal end of the ovariole (Uvarov 1966, Sundberg *et al.* 2001). Ovaries were dissected from females and both corpora lutea and resorption bodies were counted with the aid of a dissecting microscope. The mean number of eggs, corpora lutea, and resorption bodies per ovariole per individual were used in all analyses, rather than the total per individual, because the number of each of these variables could not always be determined in all ovarioles.

Statistical Analyses.— Morphometric and energetic condition in males and morphometric condition and female reproductive characteristics were compared with linear regression analyses using SYSTAT (1997). Means are reported ± one standard error ($s_{\bar{x}}$). Sample sizes for male and females varied depending on the comparison being made be-

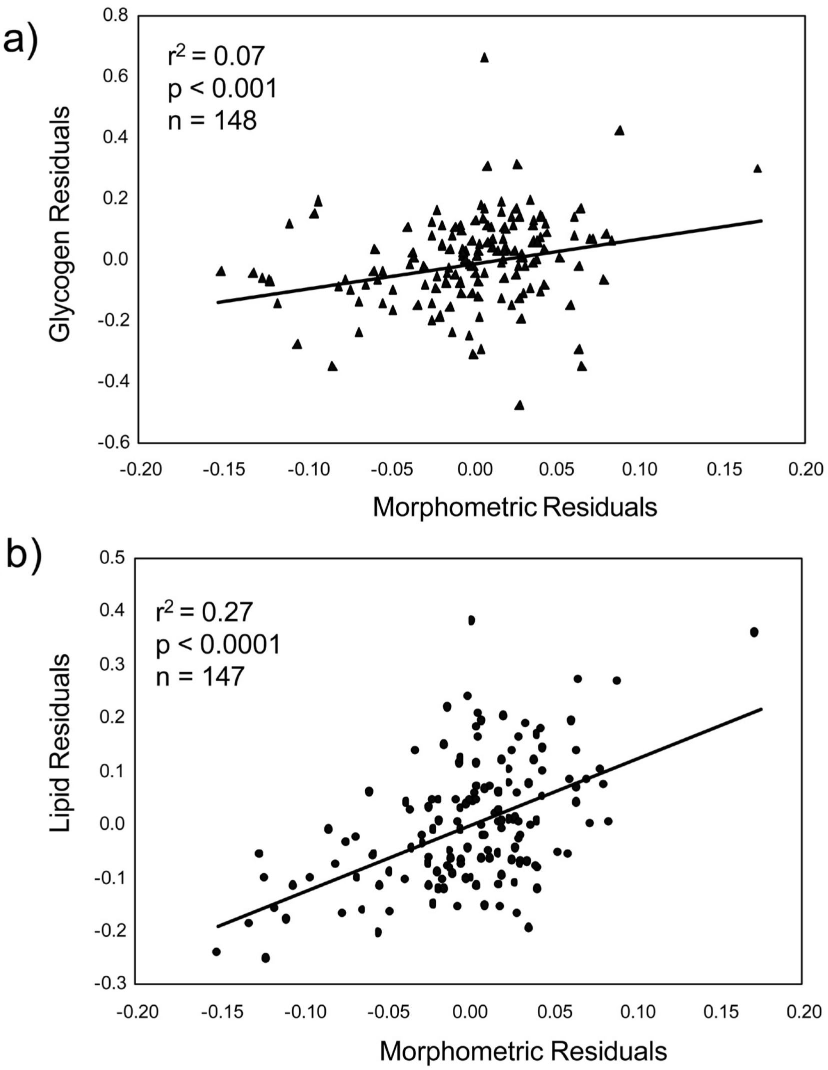

Fig. 4. Relationship between **a)** glycogen residuals and morphometric residuals (y=1.252x-0.0008) and **b)** lipid residuals and morphometric residuals (y=0.782x) for males. Glycogen and lipid residuals were taken from regressions of the $\log_{10}$ transformed quantity of those substances with $\log_{10}$ femur length. Morphometric residuals were taken from regressions of $\log_{10}$ mass with $\log_{10}$ femur length.

cause some females were missing both back legs, one lipid sample for a male was destroyed, and certain reproductive characteristics in some females were indiscernible.

Results

Size and morphometric condition in males and females.— D. variegatum is sexually dimorphic with respect to both femur length and mass (Fig.1). On average, female femur length was 11.8 ± 0.10 mm (n=59) and female mass was 0.84 ± 0.03 g (n=64); male femur length was 9.6 ± 0.04 mm (n=148) and male mass was 0.32 ± 0.004 g (n=148).

The regression of $\log_{10}$ mass *vs* $\log_{10}$ femur length for females and males showed a linear pattern (Fig. 2). Femur length explained 33% of the variation in mass of males and 24% of the variation in mass of females. The residuals of this regression represent morphometric condition.

Morphometric and energetic condition in males.— Total lipid and glycogen content in males was significantly related to femur length (Fig. 3). This relationship was weak for glycogen, with femur length explaining 8% of the variation in glycogen content, and slightly stronger for lipids, with femur length explaining 13% of the variation in lipids. Even though the relationships are relatively weak, these analyses indicate that structurally larger males have more total lipid and glycogen content than smaller males. To examine whether there is a relationship of morphometric condition and energetic condition, level of lipids and glycogen must be controlled for differences that are simply due to body size. Thus, I used the residuals of regressions of $\log_{10}$ femur length versus $\log_{10}$ glycogen content and $\log_{10}$ lipid content in the analyses comparing morphometric and energetic condition measures. Morphometric condition was significantly related to residuals of glycogen and lipids in males (Fig. 4). This relationship was weak for glycogen residuals (r^2=0.07) but stronger for lipid residuals (r^2=0.27).

Morphometric condition and female fecundity.— On average, females had 31.9 ± 0.55 ovarioles (n=60), 7.5 ± 0.13 eggs per ovariole (n=57), 0.96 ± 0.04 corpora lutea per ovariole (n=58), and 0.46 ± 0.03 resorption bodies per ovariole (n=58). There were no significant relationships between mass and: number of ovarioles (r^2=0.001, p=0.82, n=60), number of eggs per ovariole (r^2=0.00002, p=0.98, n=57), number of corpora lutea per ovariole (r^2=0.03, p=0.23, n=58). However, there was a significant relationship between mass and number of resorption bodies (r^2=0.09, p=0.02, n=58; Fig. 5). There were no significant relationships between femur length and any

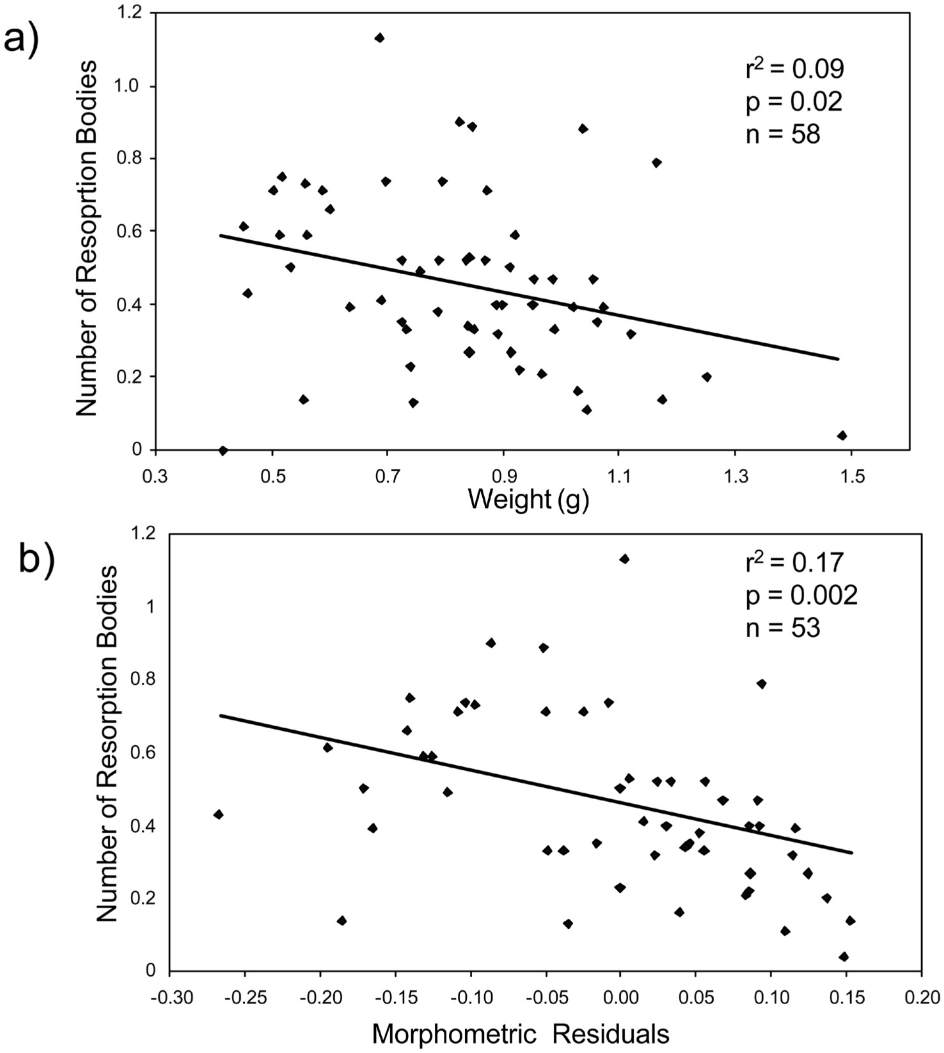

Fig. 5. Relationship between **a)** the mean number of resorption bodies per ovariole and mass/weight (g) and between **b)** the mean number of resorption bodies per ovariole and morphometric residuals. Morphometric residuals were taken from regressions of $\log_{10}$ mass with $\log_{10}$ femur length.

of the reproductive characteristics (number of ovarioles – r^2=0.01, p=0.39, n=55; number of eggs per ovariole – r^2=0.0002, p=0.93, n=52; number of corpora lutea per ovariole –r^2=0.0004, p=0.88, n=53; resorption bodies – r^2=0.02, p=0.31, n=53). There was also no relationship between morphometric condition and: number of ovarioles (r^2=0.005, p=0.62, n=55), number of eggs per ovariole (r^2=0.001, p=0.83, n=52), and number of corpora lutea per ovariole (r^2=0.04, p=0.14, n=53). However, there was a significant relationship between morphometric condition and resorption bodies (r^2=0.17, p=0.002, n=53; Fig. 5).

Discussion

D. variegatum is sexually dimorphic with respect to both mass and femur length, a common phenomenon in grasshoppers (Uvarov 1966). There was relatively little overlap between the distributions of femur length between males and females and almost no overlap in mass (Fig. 1). In addition, there was much greater variability associated with the distribution of femur length and mass for females compared to males. The presence of strong sexual dimorphism in characters related to size in *D. variegatum,* illustrates the need to analyze size-related data separately for males and females.

A major assumption of using mass-size residuals to estimate condition is that the relationship between the two variables is linear (Green 2001). Like Schulte-Hostedde *et al.* (2005), who found linear relationships between log-transformed size and mass variables in five mammalian species, I also found a linear relationship between log-transformed mass and femur length in both sexes of *D. variegatum*.

Not surprisingly, structurally larger males (*i.e.*, males with larger femur lengths) had more lipid and glycogen content, but these relationships were not as strong as expected. One possible reason for the lack of a strong relationship between structural size and total lipid and glycogen content, is that water and protein associated with muscle mass may make up a large component of total mass, and the components may vary independently of lipid and glycogen content. Regardless of the relative weakness of these relationships, the presence of significant relationships warranted controlling for structural size (*i.e.*, using the residuals of glycogen and lipids regressed against femur length) when comparing energetic condition with morphometric condition. Although past studies examining the relationship between mass-size residuals have not always controlled for differences in energy content related to body size (*e.g.*, Schulte-Hostedde *et al.* 2001 and Ardia 2005 compared total fat content with mass-size residuals), this is a necessary step if one wishes to answer the question of whether individuals with more mass loaded onto a frame (regardless of frame size) also have more energy reserves loaded onto that frame (regardless of its size).

After controlling for the effect of structural size on lipid and glycogen content, a positive relationship was found between morphometric condition and energetic condition as measured by lipid and glycogen residuals; the relationship was stronger for lipids (r^2=0.25) than for glycogen (r^2=0.07). These results may be explained by the fact that lipids are associated with long-term storage of energy, glycogen with short-term storage (Gilbert & Schneiderman 1961, Beenakkers 1969, Van Handel 1974). Glycogen levels may be more appropriate as a "snapshot" of relatively recent events affecting the energetic condition of a grasshopper, and thus not as tightly connected to morphological condition as long term energy stores, such as lipids.

This study differs from some work with mammals that suggests no relationship, or a very weak relationship, between mass-size residuals and fat reserves (*e.g.*, Krebs & Singleton 1993, Virgl & Messier 1993, Schulte-Hostedde *et al.* 2001). Schulte-Hostedde *et al.* (2001, 2005) argue that other components of body mass, such as protein and water, are more closely related to morphometric condition, and that these other components may be as important to survival and reproduction as fat reserves. Thus, Schulte-Hostedde *et al.* (2005) argue for a more inclusive definition of morphometric condition that includes its relation to all components of body composition, not just fat. They also suggest that morphometric measures of condition be evaluated against these components independently for the taxon of interest. For *D. variegatum*, it appears that morphometric condition provides some information about underlying fat stores in males. Future studies would benefit by quantifying water and protein content as well as lipids and glycogen.

In female *D. variegatum*, I found no relationship between the number of ovarioles and femur length, mass, or morphometric condition. The number of ovarioles is expected to be affected by genetic differences between individuals and/or environmental effects experienced in the maternal environment (Albrecht *et al.* 1959, Uvarov 1966, Bellinger *et al.* 1987). Either of these mechanisms could lead to a relationship of size and morphometric condition with number of ovarioles (Stauffer & Whitman 1997). Size has been found to be an important factor explaining interspecific variation in ovariole number amongst melanopline species. Bellinger and Pienkowski (1985) found a strong relationship (r^2=0.93) between ovariole number and size (as measured by femur length) among species of one genus (*Melanoplus*), with mean number of ovarioles for each species ranging between 10 and 183 per female. Using their regression equation, *D. variegatum*, with an average femur length of 11.8 mm, is expected to have 11.2 ovarioles. Although I found an average of 32 ovarioles per female, that estimate is within a standard error of Bellinger and Pienkowski's predicted value.

Although size has been suggested to be important in explaining interspecific variation in ovariole number, other evidence suggests that size does not explain intraspecific variation in ovariole numbers. Bellinger *et al.* (1987) found no relationship between body size and ovariole number within populations of *Melanoplus femurrubrum* or *M. scudderi scudderi*. Instead, for macropterous *M. femurrubrum* they found variation in ovariole number in different locations and years that was related to environmental conditions experienced by the mother and by egg size; they did not find similar variation between years in the smaller, brachypterous *M. scudderi scudderi*. Work by Bellinger *et al.* (1987) and others (Rubtzov 1934, Richards & Waloff 1954, Blackith & Blackith 1969) supports the hypothesis that relatively small brachypterous species may have reduced variation in ovariole number, possibly because these species are relatively less mobile and may be better adapted to specific environments, rather than having a great degree of plasticity. This may explain the lack of relationship between ovarioles and any measure of size or morphometric condition in *D. variegatum*. Alternatively, given the heterogeneous environment from which they were sampled, the maternal environment of the individuals collected in this study may have differed strongly from the environment in which they developed.

I also found that the mean number of eggs and corpora lutea per ovariole did not vary significantly with femur length, mass, or morphometric condition. These characteristics (mean number of eggs, corpora lutea per ovariole) may not strongly affect female fecundity in natural conditions. According to Joern and Gaines (1990) few species oviposit more than three times under natural conditions, and most individuals do not even live long enough to oviposit that many times. The average number of eggs measured for *D. variegatum* in this study was 7.5 eggs per ovariole, indicating that any minor variation in the mean number of eggs per individual probably has little impact on fitness.

Mean number of resorption bodies per ovariole was the only reproductive characteristic significantly related to mass and morphometric condition, with larger and better-conditioned females having fewer resorption bodies. The relationship was strongest with morphometric condition (r^2=0.17), suggesting that females in poor condition are more likely to reallocate energy from egg production. Because the degeneration and resorption of eggs is thought to be an important factor affecting fecundity (Uvarov 1966), resorption bodies may be a good estimator of reproductive stress (Sundberg *et al.* 2001).

In general, morphometric condition probably has limited value in estimating fitness of wild-caught females, because female mass is much more variable than male mass, as it greatly depends on the frequency and timing of oviposition. Adult female mass drops after each oviposition, then rises to a plateau before the next oviposition (Wall & Begon 1987a). Thus, within the course of a season, morphometric condition would be expected to fluctuate widely, and would not necessarily represent any useful information about the relative energetic content or reproductive fitness of the individual. The concept may be more appropriately used in the laboratory, where sampling can occur at the same time relative to an oviposition bout. In contrast, morphometric condition in males is potentially a useful tool for inferring information about energy content of grasshoppers in wild populations.

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