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Investigation of inter-individual and intraspecific allometric scaling relationships between metabolic rate and body mass in the *Manduca sexta*

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Abstract

Body mass influences many physiological characteristics of an animal. By using the *Manduca Sexta*, we can investigate the relationship between body mass and metabolic rate. Previous studies have led to disputes over the scaling ratio between them when using the mathematical relationship that says the rate is proportional to M^b, when M is the body mass of the organism and b is the scaling constant. Rubner (1883) proposed that the scaling constant should be 0.67, while the experimental values by Kleiber (1932) were 0.75. Subsequent studies have been unable to reconcile the differences between results. Here we look at the differences between both the instars and individual caterpillars while following the metabolic rates and body sizes from the 1st instar to the 5th instar. We found no difference between individuals or instars in this study, and determined a scaling ratio to be 0.84.

Introduction

One of the ways a change in body mass could affect physiological variables would be to alter metabolic rate. Metabolic rate is the rate of conversion of chemical energy to heat. For an organism, this rate is proportional to M^b, when M is the body mass and b is the exponent that relates the change in metabolic rate to the increasing body size of the animal (Schmit-Nielson, 1984).

Max Rubner, in 1883, hypothesized that an organism's metabolic rate is proportional to the surface area of its body (Randall *et al.*, 2002). When this theory is applied mathematically, the exponential value (b) should be 0.67. The underlying assumption in this theory is that the surface area increases by the square while volume increases by the cube. Experimental values, however, have shown that the exponential value should be closer to 0.75 (Kleiber, 1932) and therefore shows that Rubner's theory makes assumptions inaccurately. Since this discrepancy in theory arose, many researchers have sought to alleviate confusion on the topic and to explain why 0.75 makes more sense than 0.67 as the correct scaling exponent

$M^{0.75}$ is only applicable when comparing metabolic rates interspecifically (between species). Intraspecific scaling (within species) has not been looked at in depth and cannot be deduced from analysis of interspecific scaling (Green *et al.*, 2001) Knowledge of interspecific scaling will give us a better understanding of the phenomenon observed in interspecific scaling and even allow us to determine if it is a valid comparison to make.

An ideal model organism needs to maintain the same relative shape throughout its growth and have a large fold growth. A novel organism, the *Manduca sexta* larvae undergo five larval molts and grow rapidly, increasing in mass 10,000 fold in 16 days. During this time, the general body shape of the *M. sexta* remains the same.

Recent studies by Andrew Vreede, a 2005 graduate of Kenyon College, have found that the mass exponent between individual *M. sexta* caterpillars range from a mass exponent of 0.66 to 0.95, with a mean mass exponent of 0.82. This exponent value is significantly different from 0.67 and 0.75. An interesting find was that there were also significant interindividual y-intercepts (1.20-1.39). Overall, the y-intercepts between the instars were found to be significantly different from each other.

Methods

Animal Care:

Larval tobacco hornworm larvae (*Manduca Sexta*) were raised from eggs (Carolina Biological Supply, NC) on a prepared artificial diet (approximately 80% water, Carolina). *M. sexta* were raised in individual 16 oz. plastic containers in an incubator set at 27°C and a L:D cycle of 16h:8h. Larvae were weighed once every 24 hours during the larval stage.

Respirometry:

The scaling relationship of body size and metabolic rate of 13 actively feeding individual larvae was examined by obtaining the metabolic rate of each larvae 2 hours after the removal of food from its living chamber. This measure was preformed daily from the 2nd day of the 1st instar until approximately the 3rd day of the 5th instar. An IRGA CO2 analyzer (Qubit, Model No. S15X) subsequently measured the level of CO2 emitted from the *M. sexta* on a range from 0 ppm to 2000 ppm. After a room air baseline was measured, the larva was placed in the animal chamber and CO2 concentrations were recorded for 25 minutes.

Results

Intraspecific Scaling Relationships:

I examined the change in metabolic rate across the five instars of the *M. Sexta* larvae to gain an understanding of the association between body size and metabolic rate. Here, I constructed a log-log body size metabolic rate plot from data obtained from 13 larvae (Regression Analysis, p=0.001). The scaling constant of 0.838 was obtained when the log metabolic rate was scaled with the log body mass.

To understand the scaling constants and y-intercepts of the instars individually, the data was broken down into each instar (Figure 1; Regression Analysis). This data shows that the slopes and the y-intercepts were not significantly different between instars when all the individual larvae were grouped together (Fig 1).

Identification of Inter-Individual Variation:

To determine the inter-individual variations in metabolic rate and scaling relationships, the same log-log body size metabolic rate plot was constructed as before for each individual (four shown, Figure 2). The scaling constants ranged from 0.8463 to 0.9734 and the y-intercepts ranged from 2.059 to 2.150.
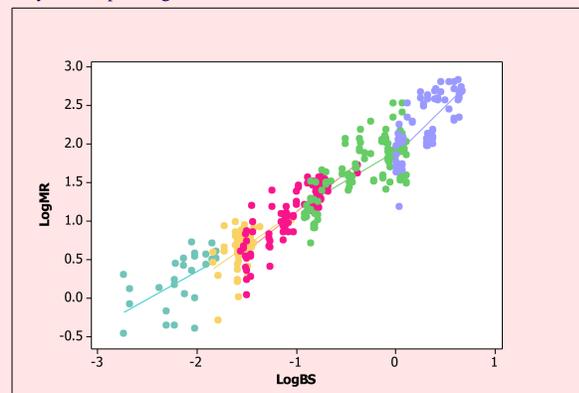

Figure 1. All data on the effect of body size on the metabolic rate of the tobacco hornworm caterpillar, *Manduca sexta* (regression analysis and Tukey's pairwise comparisons). Reg eq: $\log(MR) = 1.861 + 0.8439(\log(BM))$. $R2 = 0.893$, p= 0.000, N= 13 larvae (total measurements = 340). Teal= 1st instar; goldenrod = 2nd instar; magenta= 3rd instar; green = 4th instar; periwinckle = 5th instar.
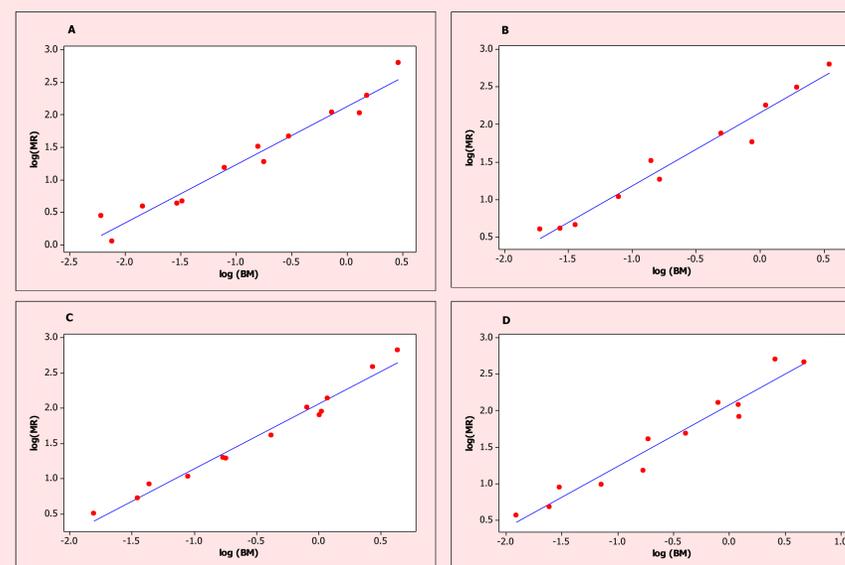

Figure 2. Individual plots of log(metabolic rate) (MR) vs. log(body mass) (BM) of *Manduca sexta* (Regression analysis and Tukey pairwise). **A**: Caterpillar 6. Reg eq: $\log(MR) = 2.130 + 0.8970(\log(BM))$. $R^2 = 0.96$, p= 0.000 , SEM= 0.228, N=1 larva (measurements=13). **B**: Caterpillar 7. Reg eq: $\log(MR) = 2.150 + 0.9734(\log(BM))$. $R^2 = 0.965$, p= 0.000 , SEM= 0.234, N= 1 larva (measurements= 11). **C**: Caterpillar 8. Reg eq: $\log(MR) = 2.059 + 0.9226(\log BM))$. $R^2 = 0.977$, p= 0.000 , SEM= 0.198, N= 1 larva (measurements= 13). **D**: Caterpillar 9. Reg eq: $\log(MR) = 2.083 + 0.8463(\log(BM))$. $R^2 = 0.951$, p= 0.000, SEM= 0.211, N= 1 larva (measurements= 12).

Methods continued…

Statistical Analysis:

All larvae were measured on three different setups in order to control for any small differences between analyses. Preliminary analysis gave the line of least squares for each analyzer and this was compared between each experimental setup using correlation statistics. Body size and metabolic rate data from the individual larvae were log-transformed and analyzed using multiple regression analysis to determine the scaling constant and y-intercept. Indicator variables containing interacting terms were utilized during the regression analysis in order to determine the y-intercepts and scaling constants between individual larvae and between each instar. An ANOVA was performed to compare the mean scaling constant and y-intercept of the individual larva. Tukey's pairwise comparisons were used to determine which of the larvae had significantly different metabolic y-intercepts and/or scaling constants (Minitab 14).

Discussion

Intraspecific Scaling Relationships

The scaling constant determined from the data presented here (0.838) was different from both predicted scaling constants: Kleibers law (0.75) and the surface hypothesis (0.67). However, this scaling constant is not abnormal when observed in the context of the observed range of intraspecific scaling constants (Bokma, 2004) along with Vreede's scaling constant. Alleyne *et al.* (1997) found the scaling constant of *M. sexta* was 0.80. Greenlee and Harrison (2005) calculated a scaling constant of 0.98. Differences in scaling constants could arise from a variety of factors, such as differences in methodologies. Greenlee and Harrison (2005) performed respirometry collections while the larvae were feeding ad libitum. Vreede's data, from his senior thesis (2005), showed a scaling constant of 0.82. The methodologies here were the same and the only difference was in the experimenter, the flow meters, and the range of body sizes where the metabolic rate was recorded.

Our hypothesis that the y-intercepts between the instars would be statistically different did not hold up during the course of this experiment (probably due to our small sample size). Comparisons between the 4th and 5th instars demonstrated the same slope but different y-intercepts. Vreede's data could explain the tracheal system growth during molts, while the body mass remained relatively the same, if not decreasing (Beitel and Krasnow, 2000).

Scaling Relationship of Inter-Individual Variation:

In the present study, there was no significant difference between the larvae chosen for individual comparisons. Previously, in our lab, it was found that there was significant variation, between individuals, in their metabolic scaling constant and the y-intercept. A possible explanation for this inconsistency could be in the type of flow meters used during the experiment. Vreede used an apparatus that was not as accurate and perhaps a smaller change in CO_2 could elicit an inaccurately large jump. The new flow meters are able to keep the flow more consistent and accurate. This may have been the cause of the variation seen in Vreede's individuals.

Acknowledgements and References

Alleyne, M., M.A. Chappell, D.B., Gelman and N.E. Beckage. 1997. Effects of parasitism by the braconid wasp *Cotesia congregate* on the metabolic rate in host larvae of the tobacco hornworm, *Manduca sexta*. Journal of Insect Physiology, 43: 143-154.

Beitel, G.L. and M.A. Krasnow. 2000. Genetic control of epithelial tube size in the *Drosophila* tracheal system. Development, 127: 3271-3282.

Bokma, F. 2004. Evidence against universal metabolic allometry. Functional Ecolgy, 18: 184-187.

Green, J., J., J. Figuerola, and R. King. 2001. Comparing interspecific and intraspecific allometry in the Anatidae. J. Ornithol., 142: 321-334.

Greenlee, K.J. and J.F. Harrison. 2005. Respirometry changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta*. Journal of Experimental Biology, 208: 1385-1392.

Kleiber, M. 1932. Body size and metabolism. Hilgardia 6: 315-353.

Schmit-Nielson, Knut. 1984. Scaling: Why is animal size so important? Cambridge University Press. Cambridge, UK.

Rubner, M.1883. Ueber den einfluss der korpergrosse auf stoffund Kraftweschel. Z. Biology, 19: 535-562.75