Disentangling determinants of egg size in the Geometridae (Lepidoptera) using an advanced phylogenetic comparative method

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Abstract

We present a phylogenetic comparative study assessing the evolutionary determinants of egg size in the moth family Geometridae. These moths were found to show a strong negative allometric relationship between egg size and maternal body size. Using recently developed comparative methods based on an Ornstein-Uhlenbeck process, we show that maternal body size explains over half the variation in egg size. However other determinants are less clear: ecological factors, previously hypothesised to affect egg size, were not found to have a considerable influence in the Geometridae. The limited role of such third factors suggests a direct causal link between egg size and body size rather than an indirect correlation mediated by some ecological factors. Notably, no large geometrid species lay small eggs. This pattern suggest that maternal body size poses a physical constraint on egg size but, within these limits, there appears to be a rather invariable selection for larger eggs.

Key Words: Egg size, Body size, Lepidoptera, Geometridae, Life history, Phylogenetic comparative methods, Ornstein-Uhlenbeck.

Introduction

Progeny size is a trait of central importance for the theory of life history evolution. For example, larger newborns, eggs, and seeds have in numerous cases been reported to show higher fitness (Braby, 1994; Williams, 1994; Fox & Czesak, 2000; Moran & Emlet, 2001; Ficetola & de Bernardi, 2009). However producing larger progeny is more costly for the mother, therefore progeny size is central to the quality-quantity trade-off that mothers have to make (Bernardo, 1996; Fox & Czesak, 2000). Currently though, despite a long history of studying this trade-off (Marshall, 1953; Salthe, 1969; Berrigan, 1991; Olsen & Cockburn, 1993; García-Barros, 1994; Kinnison *et al.,*

2001; Czesak & Fox, 2003; Forbes *et al.,* 2010), both ultimate and proximate determinants of progeny size are far from being fully understood.

Across various taxa, there exists a general pattern that larger species tend to give birth to larger offspring. At the interspecies level, correlated evolution of these traits has been demonstrated in a phylogenetic context in hymenopterans (Blackburn, 1991), some crustaceans (Poulin & Hamilton, 1997), fish (Crespi & Teo, 2002; Cooke *et al.,* 2006), frogs (Byrne *et al.,* 2003), and birds (Dyke & Kaiser, 2010). In several other taxa, a correlation between these traits has been studied but without explicitly considering phylogeny (ladybirds – Dixon & Hemptinne, 2001; whiptail lizards – Schall, 1978; turtles – van Buskirk & Crowder, 1994; rotifers – Walz *et al.,* 1995; birds – Rahn *et al.,* 1975).

For Lepidoptera in particular, despite the demonstration of allometric scaling between mother and offspring size in some groups (García-Barros & Munguira, 1997; García-Barros, 2000; 2002), quantitative and causal aspects of this relationship are still unclear. In terms of causality the allometric scaling could either 1) be based on a purely mechanistic (anatomical) constraint (maternal body must be large enough to fit large eggs), 2) a developmental constraint resulting from ontogenetic integration (genes for large body affect egg size as well), or 3) be a consequence of ecologically-based parallel selection on both egg and body size, with no causal links between the two. Maternal body size as an anatomical constraint on maximal egg size is obvious in insects such as tsetse flies, which lay a single offspring at a time, of comparable size to the mother herself (Buxton, 1955). However, the situation is less clear in species that are able to carry tens to hundreds of mature eggs simultaneously, like Lepidoptera (Wiklund & Karlsson, 1984; Wiklund *et al.,* 1987; Fox & Czesak, 2000; Bauerfeind & Fischer, 2008) and the majority of other insects (Hinton, 1981). In turn, a developmental constraint would imply that the two traits have some genetic link. Indeed, genetic correlation between offspring size and body size have been reported in other taxa (e.g. birds – Larson & Forslund, 1992; Diptera – Avezedo *et al.,* 1997), including Lepidoptera (Fischer et al.,

2002). However, the existence of a rigid developmental link between egg and body size is inconsistent with the frequent observation that egg size is not or is just weakly affected by environmentally based reductions in body size (Tammaru *et al.,* 2002; Bauerfeind & Fischer, 2008). This suggests that there is enough leeway for variation in egg-size body-size ratios (Wiklund & Karlsson, 1984).

In addition to constraint-based explanations, a positive association between egg and maternal body size could have an adaptive basis. Such a relationship may be selected for indirectly through correlation of optimal values of both egg and maternal body size with the values of some third trait, perhaps an ecological one. Indeed, it is reasonable to assume that optimal progeny size, and consequently the optimal balance between progeny size and number, will vary across different environments (Fox & Czesak, 2000). An intuitive assumption is that larger eggs give rise to more viable progeny (Smith & Fretwell, 1974), and various hypotheses citing ecological advantages of larger egg size have been established across various taxa (e.g. Palmer, 1985; Honěk, 1992; Fox, 1994; Carriere & Roff, 1995). Consistent with this basic assumption, lepidopteran species with larger offspring (from larger eggs) have been shown to be able to feed on a wider range of host plants, including those less favourable (Wasserman & Mitter, 1978; Nakasuji, 1987; Braby, 1994). Additionally, in polyphagous species where females are less discriminating about laying their eggs on particular host plants, it is advantageous for offspring to be able to disperse further afield (Tammaru *et al*., 1995). Larger offspring have also been shown to disperse further (Wellington, 1965; Barbosa & Capinera, 1978). Furthermore for offspring which feed on tougher woody plants as opposed to softer herbaceous plants, it has been shown that those feeding on the former come from larger eggs (Reavey, 1992). Also, host plants are considered to be more numerous and more easily found in forest habitats compared to non-forest environments, which necessarily bears consequences for various life history traits (Barbosa *et al.,* 1989; Snäll *et al.,* 2007). Environmental factors, which could determine egg size are not necessarily linked to diet or habitat. For example, it

is also hypothesised that larger eggs are a survival mechanism in species that overwinter in this stage (Reavey, 1992).

Previous studies in selected groups of Lepidoptera, which consider egg size and maternal body size in a phylogenetic context (e.g. García-Barros, 2000; 2002) have been limited to the commonly-applied comparative method of phylogenetic independent contrasts (Felsenstein, 1985). Such analyses cannot go beyond testing correlated evolution. In this study we use a recently developed method of phylogenetic comparative analysis based on an Ornstein-Uhlenbeck model of trait evolution, which enables us to examine potential adaptive relationships of traits, involving directed movement (though there is also a stochastic component) towards specific optimal values. These optima can themselves evolve on the phylogeny and can be modeled on categorical factors or continuous measurements of the selective environment (Hansen, 1997; Butler & King, 2004; Hansen *et al.,* 2008; Labra *et al.,* 2009).

The primary aim of this study is to understand the factors that underlie variation in egg size in geometrid moths, using an original 53-species data set. We chose the Geometridae as our study group, because they display large variation in maternal body size, egg size and ecological habit. The life history of geometrid moths is well documented and is now starting to be understood in a phylogenetic context (Snäll *et al.,* 2007; Wahlberg *et al.,* 2010), though egg size has never been systematically studied in an evolutionary ecology framework. Here we test the hypotheses that maternal body size variation is a potential determinant of egg size variation, along with four external ecological factors. These ecological factors are tested to see if they have affect on egg size either directly, or possibly indirectly (via influencing maternal body size). These factors are larval host range (poly- vs oligophagous species), tree- vs herb feeding in larvae, habitat preference (forest vs non-forest species), and overwintering stage (eggs or not).

Materials and methods

Study organisms

The Geometridae are a well characterised, distinct monophyletic group of Lepidoptera (Minet & Scoble, 1999; Hausmann, 2001; 2004). Nevertheless, there is considerable variation in life history traits (e.g. Barbosa *et al.,* 1989; Snäll *et al.*, 2007; Javoiš *et al.*, 2011) including body and egg sizes (Brehm & Fiedler, 2004), as well as in habitat choice, dietary habit (polyphagous or oligo/monophagous, tree or herb diet) and overwintering stage (Koch, 1984; Leraut, 2009). Such a high ecological diversity makes this group very attractive for studying correlates and potential determinants of egg size (or other life history traits) in a phylogenetic comparative framework.

Phylogeny

For constructing a phylogenetic hypothesis (Figure 1), we principally used DNA sequence data for Geometridae provided by Snäll *et al.,* (2007), Viidalepp *et al.,* (2007), Õunap *et al.,* (2008), Wahlberg *et al.,* (2010) and Õunap *et al.,* (2011), but also a small portion of unpublished original sequences. The final data matrix comprised 116 species and 4643 positions, and the tree was constructed in BEAST (Drummond & Rambaut, 2007) using Bayesian inference. Since results of comparative phylogenetic analyses are more easily interpreted if ultrametric trees are used (Hansen, 1997; Butler & King, 2004; Hansen *et al.,* 2008), we constructed an ultrametric tree using BEAST 1.5.4 (Drummond & Rambaut, 2007). The 116 species tree was constructed as part of some ongoing research into geometrid phylogeny at the University of Tartu. For the purposes of the present analysis, the tree was pruned to leave just the 53 relevant species for which we had life history data. Further details of the phylogenetic analysis can be found in supplementary material.

Life history data

To obtain data on maternal body and egg sizes, the moths were reared in the laboratory on their preferred host plants. Larvae were either collected from the field, or adult females were collected with their offspring being reared in the laboratory. A day after adult eclosion, each upreared female was killed by freezing, air-dried in the lab (23°C) and weighed. The abdomens were then soaked in water and dissected. Lengths and widths of three mature (chorionated) eggs were recorded for each individual.

Sample sizes of female individuals per species ranged from 1-48 (see Figure 1) depending on how many specimens could be reared. Dry weight of the freshly eclosed adults served as the measure of body size in the analyses. Using insects reared in standard laboratory conditions and of fixed adult age minimises environmentally induced variation in egg and body size (García-Barros, 1992; Oberhauser, 1997; Fischer *et al.,* 2003). Egg size was calculated as the volume of a prolate ellipsoid based on measurements of egg length and width as consistent with egg shape in geometrid moths (Johnson *et al.,* 1987). For each individual, the mean value of three measured eggs served as the estimate of egg size.

Data for habitat and larval diet was obtained from Leraut (2009), and data for overwintering stage was taken from Koch (1984). All ecological variables were coded in a binary form. Species were designated as being either oligophagous or polyphagous, and also as either tree or herb feeders. We considered oligophagy in a strict sense to indicate that a particular species feeds on just two specific genera of plants at most. Habitat preference was split into forest and non-forest dwelling species. Values for life history traits are given in Figure 1.

Phylogenetic comparative analyses

To explore the determinants of egg size, we performed the comparative analysis using the "SLOUCH" package (Hansen *et al.,* 2008; Labra *et al.,* 2009) written for the R statistical

environment (2005). SLOUCH performs phylogenetic comparative analyses based on an Ornstein-Uhlenbeck (OU) model of evolution in a maximum likelihood framework. The OU-based method enables us to model the evolution of a trait across a phylogenetic tree in response to different selective regimes (Hansen, 1997; Butler & King, 2004; Hansen *et al*., 2008). These selective regimes themselves can be values or states of other traits, or those of the environment (predictor variables). SLOUCH works with continuous response traits whereas predictor variables can be both continuous and discrete (Hansen *et al*., 2008).

When studying a regression relationship between two continuous traits – egg size and maternal body size in the present case – it must be decided which one of these traits should be treated as a response or dependent variable, and which one as the predictor or independent variable. To do this we examined the strength of the phylogenetic signal in each of the traits taken separately: indeed, it makes sense that a trait exhibiting a weak phylogenetic signal would be more free to vary in response to changes in the state of the other trait (Hansen *et al.,* 2008; Labra *et al.*, 2009). The strength of the phylogenetic signal is captured by a metric termed phylogenetic half-life (when the SLOUCH regression is performed with only an intercept (i.e. no predictor variables)), being expressed relative to the height of the tree (Labra *et al.,* 2009). Accordingly, following Hansen et al. (2008) and Labra et al. (2009), the trait exhibiting a smaller half-life is identified as the response variable. The underlying logic is as follows: 1) if one trait evolves quickly, producing an essentially random pattern with respect to phylogenetic relationships and the other is strongly influenced by phylogenetic proximity, an observed strong correlation between the traits cannot be due to a shared phylogeny; 2) this leaves us with the possibilities that the observed correlation is generated by either a) both traits responding to a hidden third variable, b) the traits responding to each other, or c) one trait being dependent on changes in the other; 3) it is however, highly unlikely that a trait responding to changes in another, randomly evolving trait with respect to the phylogeny, would generate a strong phylogenetic signal in the responding trait by chance and, 4) therefore, if there is a

causal relationship between the variables, it is more likely that the trait that is more free to vary with respect to the phylogeny is responding to changes in the phylogenetically constrained trait. Naturally, causality cannot be rigorously proved in a macroevolutionary framework and there is no way to discount the influence of all possible "hidden third variables", but these methods provide a means to make educated inferences regarding the directionality of possible causal relationships.

When a trait exhibits phylogenetic signal it is not immediately clear whether this is due to the trait itself having strong inertia, or if the trait is evolving in response to a phylogenetically structured variable. Since we also obtain a measure of half-life for a response trait when testing it against a predictor, it is possible to examine which of these two phenomena underlies the phylogenetic signal in the trait. When a trait rapidly evolves in response to a phylogenetically-structured variable (i.e. that which we test as a predictor), we may see a reduction in the half-life for the response trait as compared to the phylogenetic signal measured when we do not include the predictor in the regression (see Labra et al, 2009 for further explanations of this point).

SLOUCH analyses also consider standard errors of the input variables. For single sample species (i.e. for which standard errors could not be calculated) we took a conservative approach using the largest observed standard errors for other species. The largest standard error for (log) maternal body size in our data set is that of *Lycia pomonaria* (0.71), so we set the standard error for single sample species to 0.71. For (log) egg size, the largest standard error is that of *Gymnoscelis rufifasciata* (0.35), so we set the standard error for single sample species to 0.35.

SLOUCH returns an estimate of the similarity between the observed regression, or evolutionary regression (see Labra *et al.,* 2009) between our response and predictor variables is to the so-called optimal regression. The optimal regression is the expected scenario in the hypothetical case when phylogenetic inertia in our response trait was absent (half-life = 0) (Hansen, 1997; Hansen et al., 2008; Labra *et al.,* 2009). Comparing observed and optimal regressions indicates

how close a response trait is to its optimal state, and provides further indication as to how rapidly this response trait evolves.

Following the bivariate analysis of body and egg size, we modeled egg size as a dependent response variable against categorical ecological predictor variables (diet, habitat, overwintering stage). In order to test the influence of such ecological traits, the evolutionary histories of these ecological traits must be mapped on the phylogeny (Hansen, 1997; Butler & King, 2004). The evolutionary histories of all categorical variables were estimated by individually reconstructing them from the tip to the root of the tree using Fitch parsimony, allowing character state reversals along lineages (Fitch, 1971). To evaluate the robustness of our results, both accelerated and delayed transformation optimisation (ACCTRAN and DELTRAN (Swofford & Maddison, 1987)) of character states were tested. A third method, maximum likelihood ancestral state reconstructions, (a more informed approach using phylogenetic branch length information) (Pagel, 1999; Davis *et al.,* 2009) cannot be applied to mixed predictor models with continuous predictors because of current software limitations not allowing us to use ambiguous character states at nodes. In any case, the resulting maximum likelihood reconstructions we made range from 88-92% similar, depending on the variable, to those reconstructed using parsimony and we do not expect that using maximum likelihood reconstructions would seriously alter the overall results of the analyses presented here.

All categorical variables were tested individually as predictors of egg size to see if they could account for any of the variation observed in this trait. They were further tested as predictors against maternal body size as well to determine if these variables are influencing maternal body sizes (i.e. is any phylogenetic signal seen in maternal body size explained by diet, habitat or overwintering state). In the present context, this was primarily important in order to evaluate the possibility that any covariation between maternal body size on egg size may be the result of a parallel adaptation of both these traits to particular selective environments.

We also wished to examine the effect of combinations of predictors, as the underlying influences in variation in egg size are widely acknowledged to be complex (e.g. Bernardo, 1996; Fox & Czesak, 2000). We might expect combinations of unrelated predictors to explain more of the variance in egg size than a single predictor alone (Labra *et al.,* 2009). We therefore also ran models with combinations of body size, as a randomly changing, continuous predictor variable with individual fixed, categorical variables).

We compare how well different models explain the evolution of a response trait based on Akaike's Information Criterion (AIC), as suggested by Butler & King (2004), supported by Freckleton (2009), and following Labra *et al.,* (2009). Here we use AICc, which is a small sample adjustment of AIC (Burnham & Anderson, 1998). The variance explained for a given model is also provided by SLOUCH as a phylogenetically corrected R^2 value.

Results

An important initial finding is that egg size exhibits very little phylogenetic signal (best model – 1.3% tree height), whereas we found evidence of considerable phylogenetic signal in maternal body size (best model – 21.9% tree height). Consequently, it is clearly more meaningful to model egg size as a response to optima influenced by maternal body size rather than vice versa (see materials and methods).

The best performing model explaining egg size evolution was found with maternal body size as a lone predictor (AICc = 80.121). The r^2 of 56% indicates that maternal body size explains more than half of the variation in egg size optima. The best estimate of the evolutionary regression between egg and maternal body size shows a negative allometric relationship of egg size (log) on maternal body size (log) (slope 0.65 (SE 0.09), intercept -5.35 (SE 0.42), which implies a negative allometric relationship of egg size on body size. The optimal regression of log egg size on log

maternal body size is almost identical to the observed regression, indicating that egg sizes are very close to their optimal states (Figure 2). Other candidate models using single discrete predictors do not explain the variation in egg size so well and return much lower r^2. Combining predictors (i.e. maternal body size plus one discrete variable) does not increase r^2 substantially. For r^2 values and AICc scores see Table 1.

When maternal body size was treated as the response variable against ecological predictors there are some large reductions in half life with particular predictors (polyphagy vs oligophagy ACCTRAN model – 3.2% tree height, tree vs herb diet ACCTRAN model – 2.1% tree height) and some smaller reductions with others (see Table 1). This could indicate that any phylogenetic signal we see in maternal body size is due to phylogenetic signal in these predictors. However, r^2 values for best models indicate very little influence of these predictors on maternal body size. High AICc scores (i.e. low support) for these models support this claim (Table 1). Furthermore, where ACCTRAN models show obvious reductions in half-lives with diet-related predictors, DELTRAN models do not (Table 1). We believe that our results do not provide enough evidence against maternal body size being a phylogenetically inert trait. This scenario is also supported by some previous evidence, which shows body size in various taxa to exhibit substantial phylogenetic inertia (Diniz-Filho *et al.,* 1998; Blomberg *et al.,* 2003).

Discussion

We observed a positive correlation between maternal body size and egg size in geometrid moths. The relationship was found to be negatively allometric which is in agreement with the pattern found in other phylogenetic comparative studies of Lepidoptera (García-Barros & Munguira, 1997; García-Barros, 2000), spiders (Marshall & Gittleman, 1994), birds (Olsen & Cockburn, 1993), and lizards (Bauwens & Díaz-Uriarte, 1997), perhaps demonstrating this as a

widespread phenomenon. Quantitative comparisons across taxa are currently complicated by methodological differences among different studies, some of which do not consider phylogenetic effects (Rahn *et al.,* 1975; Hines, 1982; Berrigan, 1991; Ryan & Lindemann, 2007). The increasing availability of phylogenies and respective computational methods allows one to expect rapid progress along these lines.

The current study goes beyond just showing a positively correlated evolution of maternal body size and egg size. Having calculated phylogenetic half-lives of the two traits (much higher in body size than egg size) we can infer the likely direction of any evolutionary causality: egg size adapts to changes in body size and not vice versa. To our knowledge, for egg size and maternal body size, the causal direction of any potential adaptive relationship has never been explicitly tested before, and been just routinely assumed (Bernardo, 1996). Making this assumption, i.e. defining egg size as the response variable enabled us to examine the relationship between these traits using the Ornstein-Uhlenbeck process-based comparative methods. The results indicate that evolutionary changes in maternal body size exert a large influence on the variation we see in egg size in this group (responsible for over half the variation). Since we estimated a largely different magnitude of the phylogenetic signals for these two traits we can also make the argument that the observed allometry is not related to a common underlying genetic mechanism, and therefore the hypothesis regarding genetic constraint appears unlikely. This is consistent with weak or no within-species phenotypic correlations between the two traits found in some other lepidopterans (Wiklund *et al.,* 1987; Tammaru *et al.,* 2002; Bauerfeind & Fischer, 2008).

Furthermore, we find no evidence that the evolution of egg size or maternal body size is strongly related to ecological factors previously hypothesised to affect optima for these traits: there was no effect of larval host range, tree- vs herb feeding in larvae, habitat or overwintering stage. Therefore we tend to exclude the possibility that the relationship we see between maternal body size and egg size is indirect, (i.e. mediated by an independent adaptation of both of these traits to a third

factor). Not only does this rule out that these factors strongly influence egg size variation, but it also gives no support for respective hypotheses suggesting that they influence body size in Lepidoptera (e.g. Nakasuji, 1987; Reaves, 1992; Braby, 1994). Combining maternal body size as a predictor of egg size with independent ecological predictors does not explain much extra variation in egg size than does maternal body size alone.

Since maternal body size explains just 56% of the variation in egg size selective factors other than those considered in this study should play a role. A promising path may be to relate egg size to egg production strategies (capital vs income breeding, pro- vs synovigeny: Jervis & Ferns, 2004; Javoiš *et al.*, 2011), but such analyses are currently hindered by scarcity of comparable data available for a sufficient number of species. Whether multiple ecological factors in combination play a larger role in determining egg size variation cannot be tested due to current software limitations and testing this must wait until the relevant software becomes available.

Without any evidence for another factor playing a major role in egg size variation, we must ask how maternal body size determines egg size. Our finding that the phylogenetic half-lives of these traits are markedly different suggests that egg size changes rapidly follow maternal body size changes. The closeness of the actual regression of the values of egg size on maternal body size to the optimal regression (Figure 2) further demonstrates that egg size is a highly responsive trait. Although the comparative methods based on an Ornstein-Uhlenbeck model of evolution were designed to model adaptive evolution (Hansen, 1997; Butler & King, 2004; Hansen *et al.,* 2008), hypotheses of constraint (in this context: evolutionary forces which are not ecological in their nature) should always be considered as an alternative explanation to those of adaptation (Maynard Smith *et al.,* 1995). Rather than egg size changes occurring primarily as an adaptation to the external environment, our results suggest that maternal body size acts as an anatomical constraint that affects the adaptive optima for egg size. In other words, it may be complicated for small moths to lay large eggs for reasons which are not ecological in their nature. When maternal body size

increases (and therefore the constraint is lessened), egg size is further able to increase and rapidly follows suit.

Indeed, it is rather intuitive to see that some anatomical and physiological constraints on large eggs in small insects are inevitable (e.g. caused by limits on size of some part of the reproductive organs, etc.). Such constraints would explain why, in our study, the top left corner of the graph in Figure 2 has no data points. However, it is much less clear why some large mothers do not lay a high number of small eggs (i.e. why is the lower right corner of Figure 2 devoid of data points?). If egg size was driven by external ecological selective factors to a considerable extent, and given the ecological diversity of geometrid moths, it should be reasonable to assume that the optimal solution to the offspring quality (size)-quantity trade-off should sometimes substantially differ, even for similarly sized moths. As this appears not to be the case, we are inclined to infer that there is some rather universal ecologically based selection towards larger egg size in geometrid moths: it is advantageous to lay as large eggs as permitted by maternal body size. The exact nature of the involved selective forces remains to be elucidated.

On a broad scale, this study indicates the potential of the most recently developed OU-based phylogenetic comparative methods to enhance our understanding of relationships between life history traits. With the novel and rapidly developing OU-based methods, we can begin to understand the nature of the phylogenetic signal observed in traits of interest, (i.e. which of those respond quickly (be that adaptation to the environment or following some constraining physiological factor) or slowly (e.g. due to phylogenetic inertia), and consider the extent of adaptive or constraint-based relationships between life history traits. We eagerly await extensions to currently available software which will allow more in depth exploration of the role of categorical variables, which in the current context will allow us to assess in greater depth the role of ecological habit on life history evolution.

Acknowledgements

Table/Figure legends

Table 1 – Individual trait tests for phylogenetic signal in egg size and maternal body size (bold), and optimal regressions of different variables on egg size and maternal body size. A and D = ACCTRAN and DELTRAN character optimisations respectively.

Figure 1 – Ultrametricised phylogeny of the 53 species of geometrid moths used in this study. Relative (%) and absolute (time) scale bars indicating tree height below shown. Sample sizes and life history trait values are provided for all species.

Figure 2 – Optimal (dashed line) v observed evolutionary (dotted line) regressions of egg size on maternal body size. Optimal and evolutionary regressions are shown. The variance explained in egg size by maternal body size is $R = 56.6\%$. We interpret that the absence of points in the top left corner indicates constraint imposed by maternal body size and the absence of points in the lower right corner should be a result of ecologically based selection against small eggs in larger moths.

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Trait (in bold) + Predictors	Half-life: % tree height (t1/2) [support region]	R^2	AICc
Egg Size	1.3 [0.0-22.4]	-	-
+ Maternal Body Size	0.4 [0.0-21.2]	56.6	80.121
+ Diet (Poly/Oligophagy)	3.0 [0.0-21.2] (A) 1.1 [0.0-23.4] (D)	0.45 (A) 2.2 (D)	118.055 (A) 116.496 (D)
+ Diet (Tree/Herb)	4.0 [0.0-19.1] (A) 5.1 [0.0-19.1] (D)	9.12 (A) 8.09 (D)	114.357 (A) 112.588 (D)
+ Habitat	5.1 [0.0-21.2] (A & D)	8.3 (A & D)	112.462 (A & D)
+ Overwintering Eggs	1.1 [0.0-21.2] (A & D)	0.01 (A & D)	116.727 (A & D)
+ Maternal Body Size & Diet (Poly/Oligophagy)	0.4 [0.0-17.0] (D)	56.5 (D)	82.820 (D)
+ Maternal Body Size & Diet (Tree/Herb)	1.7 [0.0-17.0] (D)	58.76 (D)	80.555 (D)
+ Maternal Body Size & Habitat	0.4 [0.0-17.9] (A & D)	57.2 (A & D)	81.94015 (A & D)
+ Maternal Body Size & Overwintering Eggs	0.4 [0.0-19.1] (A & D)	56.66 (A & D)	82.744 (A & D)
Maternal Body Size	21.9 [0.0-85.1]	-	-
+ Diet (Poly/Oligophagy)	3.2 [0.0-44.7] (A) 22.3 [0.0-INF] (D)	7.5 (A) 0.15 (D)	135.73 (A) 136.084 (D)
+ Diet (Tree/Herb)	2.1 [0.0-46.8] (A) 18.1 [0.0-INF] (D)	6.8 (A) 02.24 (D)	136.125 (A) 135.099 (D)
+ Habitat	16.0 [0.0-57.4] (A & D)	6.6 (A & D)	132.878 (A & D)
+ Overwintering Eggs	21.9 [0.0-INF] (A & D)	3.16 (A & D)	134.510 (A & D)

	Sample Size (Body/Egg)	Maternal Body Size (10^{-4}g) (Log Maternal Body Size)	Egg Volume (mm^3) (Log Egg Volume)	Polyphagy(P)/ Oligophagy(O)	Tree feeder(T)/ Herb feeder(H)	Forest(F)/ Non-forest(N)	Overwinter eggs? Yes(Y)/No(N)
Trichopteryx carpinata	5/5	106.40 (4.67)	0.11 (-2.20)	O	T	F	N
Gymnoscelis rufifasciata	8/8	36.00 (3.58)	0.04 (-3.21)	P	H	N	N
Eupithecia vigaureata	19/17	41.42 (3.72)	0.05 (-2.95)	P	H	N	N
Hydrelia flammeolaria	1/1	30.00 (3.40)	0.04 (-3.16)	P	T	F	N
Horisme tersata	5/5	102.00 (4.62)	0.15 (-1.92)	O	H	F	N
Rheumaptera undulata	2/1	143.00 (4.96)	0.07 (-2.71)	O	T	F	N
Operophtera brumata	20/20	64.20 (4.16)	0.10 (-2.29)	P	T	F	Y
Epirrita autumnata	6/6	152.50 (5.03)	0.22 (-1.51)	P	T	F	Y
Mesoleuca albicillata	1/1	166.00 (5.11)	0.15 (-1.88)	O	H	N	N
Perlurga comitata	6/6	145.67 (4.98)	0.06 (-2.88)	O	H	N	N
Eulithis pyropata	5/5	225.40 (5.42)	0.18 (-1.72)	O	H	N	Y
Ecliptoptera silaceata	6/3	125.17 (4.83)	0.23 (-1.46)	O	H	F	N
Epirrhoe tristata	9/9	95.44 (4.56)	0.10 (-2.30)	O	H	N	N
Epirrhoe alternata	48/40	81.35 (4.40)	0.10 (-2.34)	O	H	N	N
Xanthorhoe biriviata	5/2	73.20 (4.29)	0.10 (-2.35)	O	H	N	N
Xanthorhoe ferrugata	2/2	62.50 (4.14)	0.11 (-2.19)	P	H	N	N
Xanthorhoe spadiceara	13/13	82.69 (4.42)	0.07 (-2.62)	P	H	N	N
Cyclophora albipunctata	1/1	70.00 (4.25)	0.05 (-3.00)	O	T	F	N
Cyclophora annularia	2/1	78.00 (4.36)	0.05 (-3.01)	O	T	F	N
Jodis putata	18/18	49.94 (3.91)	0.05 (-3.03)	O	H	N	N
Crocallis elinguaria	1/1	398.00 (5.99)	0.48 (-0.74)	P	T	F	N
Ennomos autumnaria	12/12	687.25 (6.53)	0.43 (-0.84)	P	T	F	Y
Ennomos fuscantaria	1/1	476.00 (6.17)	0.30 (-1.20)	P	T	F	Y
Colotois pennaria	3/3	709.67 (6.56)	0.40 (-0.91)	P	T	F	Y
Lomographa temerata	14/14	115.79 (4.75)	0.07 (-2.69)	P	T	N	N
Cabera pusaria	2/2	106.50 (4.67)	0.11 (-2.23)	P	T	F	N
Cabera exanthemata	9/9	108.56 (4.69)	0.08 (-2.58)	P	T	F	N
Plagodis pulveraria	4/4	191.25 (5.25)	0.14 (-1.93)	P	T	F	N
Pseudopanthera macularia	2/2	53.50 (3.98)	0.09 (-2.40)	P	H	F	N
Cepphis advenaria	13/13	98.08 (4.59)	0.11 (-2.24)	P	H	N	N
Selenia tetralunaria	24/24	424.21 (6.05)	0.50 (-0.70)	P	T	F	N
Selenia dentaria	5/5	360.00 (5.89)	0.29 (-1.25)	P	T	F	N
Lomaspilis marginata	10/9	54.30 (3.99)	0.05 (-2.99)	O	T	F	N
Abraxas grossulariata	6/6	375.50 (5.93)	0.57 (-0.56)	P	H	N	N
Calospilos sylvata	2/2	172.50 (5.15)	0.16 (-1.83)	O	T	F	N
Semiothisa clathrata	24/23	59.92 (4.09)	0.04 (-3.15)	O	H	N	N
Semiothisa liturata	5/5	135.20 (4.91)	0.09 (-2.40)	P	T	F	N
Semiothisa notata	2/2	108.00 (4.68)	0.07 (-2.59)	P	T	F	N
Itame brunnearia	2/2	50.50 (3.92)	0.10 (-2.28)	O	H	N	Y
Itame wauaria	2/2	145.00 (4.98)	0.15 (-1.93)	O	H	F	Y
Aethalura punctulata	16/9	102.94 (4.63)	0.08 (-2.58)	O	T	F	N
Ectropis crepuscularia	1/1	283.00 (5.65)	0.11 (-2.19)	P	T	F	N
Cleora cinctaria	9/8	174.33 (5.16)	0.09 (-2.44)	P	H	N	N
Lycia hirtaria	5/5	1506.00 (7.32)	0.19 (-1.64)	P	T	F	N
Lycia pomonaria	2/1	387.00 (5.96)	0.18 (-1.70)	P	T	F	N
Agriopis aurantiaria	4/4	146.00 (4.98)	0.16 (-1.82)	P	T	F	Y
Arichanna melanaria	13/13	207.54 (5.34)	0.27 (-1.30)	O	H	F	N
Erannis defoliaria	8/8	365.63 (5.90)	0.22 (-1.53)	P	T	F	Y
Paradarisa consonaria	15/14	202.33 (5.31)	0.09 (-2.42)	P	T	F	N
Alcis repandata	1/1	282.00 (5.64)	0.10 (-2.32)	P	T	F	N
Phigalia pedaria	1/1	436.00 (6.08)	0.14 (-1.97)	P	T	F	N
Ematurga atomaria	15/15	115.60 (4.75)	0.08 (-2.47)	P	H	N	N
Hypomecis punctinalis	9/9	469.89 (6.15)	0.09 (-2.37)	P	T	F	N

100%   0%

40 30 20 10 0

Millions of years

SUPPLEMENTARY MATERIAL – PHYLOGENETIC ANALYSIS

Sequence data form one mitochondrial protein-coding gene (*COI*), one nuclear rRNA (*28S rRNA*, expansion segments D1 and D2) gene and five nuclear protein-coding genes (*EF-1α*, *wgl*, *RpS5*, *IDH*, *GAPDH*) were used to construct an underlying phylogenetic framework used in the phylogenetic comparative analyses. The majority of the data has been published earlier by several authors (Snäll *et al.,* 2007, Viidalepp *et al.* 2007, Õunap *et al.,* 2008, 2011, Wahlberg *et al.,* 2010), but a small subsample of original unpublished information (reaction protocols and methods of further treatment of data have been provided in Õunap et al, 2011) was also used. Downloaded and original sequences were aligned using CLUSTALW (Thompson et al., 1994) with default settings in BIOEDIT (Hall, 1999). No indel events were found in *COI*, *EF-1α*, *GAPDH* and *IDH* alignments, so alignment was simplified. However, a few taxon-specific patterns were discovered in the cases of *wgl* and *RpS5*. All Larentiinae taxa had a unique 6-9 bp insertion in the *wgl* sequence and a deletion of 3 bp in the *RpS5* sequence, respectively. As these indels were discovered in alignments with no more ambiguities, the respective sequences were not further modified prior to the phylogenetic analysis. The lengths of the fragments of protein-coding genes used in the phylogenetic analysis were 666 bp for *COI*, 973 bp for *EF-1α*, 398 bp for *wgl*, 691 bp for *GAPDH*, 699 bp for *IDH* and 617 bp for *RpS5*. Alignment of the 28S fragments proved more difficult, as had been shown earlier by several authors (e.g. Snäll et al., 2007, Õunap et al., 2008, Wahlberg et al., 2010). The length of successfully sequenced fragments of D1 varied from 280-281 bp and the length of aligned data matrix was 283 bp. Seven positions with indels were excluded from data matrix resulting in a 276 bp indel-free matrix. The alignment of D2 was even more complicated, as the length of successfully sequenced fragments varied from 296-437 bp and the length of aligned data matrix was 476 bp. Of those positions 153 contained indels and were removed, resulting in a 323 bp indel-free data matrix. The total length of the combined data matrix was 4643 bp.

For phylogenetic analysis, the data were divided into three partitions. First, *COI* as the single mitochondrial protein-coding gene was treated as a separate partition. Second, as expansion segments D1 and D2 of *28S* are different regions of the same rRNA gene and therefore share a similar evolutionary history, data from these gene fragments were concatenated and treated as a single partition. Third, as sequencing nuclear protein-coding genes proved difficult and failed on a number of occasions (see Wahlberg et al, 2010, Õunap et al, 2011), the data from all respective gene fragments were treated as one partition and failed regions were defined as missing characters. MODELTEST 3.06 (Posada & Crandall, 1998) was used in PAUP*4.0b10 (Swofford, 1998) to search for the model of DNA substitution that best fitted the data for each partition. BEAST v1.5.4 (Drummond & Rambaut, 2007) was used for the Bayesian estimation of phylogeny, implementing GTR+I+Γ model selected by Modeltest for each of the tree partitions and using relaxed molecular clock allowing branch lengths to vary according to an uncorrelated lognormal distribution (Drummond et al, 2006). To obtain an ultrametric tree, the age of the Geometridae was calibrated according to Yamamoto & Sota (2007), i.e. 54.4 million years with a standard deviation of 4 million years. The MRCA of *Hypomecis+Ematurga* clade was given a uniform prior distribution from 4 to 10 million years according to Wahlberg et al (2010).

The phylogenetic analysis was performed using BEAST 1.5.4 (Drummond & Rambaut, 2007). Bayesian MCMC was run over 40 million generations, sampling every 1000th generation. Two independent runs were performed starting with automatically generated random number seeds. The results were inspected with TRACER v1.5 and the point of convergence was determined visually for both runs. All trees sampled prior to these points of convergence were discarded as ‚burn-in'. Summary trees were generated using remaining trees in TREEANNOTATOR v1.5.4 and the results were visualized with FIGTREE v1.3.1. Thereafter, the results of both runs were compared. As both analyses resulted with an identical tree topology, we concluded that these results represent a global optimum. For phylogenetic comparative analysis we selected summary tree from the run that had converged on a stationary level earlier. Resulting 116-taxon tree was pruned, retaining

only the 53 taxa for which we had life history data. The height of the final

tree is 47 million years (Figure 1), which is younger than recently indicated by

Yamamoto & Sota (2007) and Wahlberg et al (2010) but within the 95% confidence

intervals for divergence times according to the latter, more recent analysis. It is important to note

though that in the comparative analyses that follow, it is the relative height of the tree and not the

absolute height which is important, and all results will be discussed in the relative context.

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