Greater host breadth still not associated with increased diversification rate in the Nymphalidae—A response to Janz et al.

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In their technical comment, Janz et al. take issue with our recent study examining the association between host breadth and diversification rates in the brush-footed butterflies (Lepidoptera: Nymphalidae) (Hamm and Fordyce 2015). Specifically, they are concerned that we misrepresent their "oscillation hypothesis" (OH) (Janz et al. 2006; Janz and Nylin 2008) and that one of our models was inadequate to test hypotheses regarding host breadth and diversification rate. Given our mutual interests in the macroevolutionary patterns of herbivorous insects, we appreciate the opportunity to respond to their concerns.

The Oscillation Hypothesis and Hypothesis testing

What began as a compelling hypothesis to explain host range evolution (Janz et al. 2001) has grown to encompass biogeography and speciation (Slove and Janz 2011; Janz and Nylin 2008), phenotypic plasticity, and physiology (Janz et al. 2006; Nylin and Wahlberg 2008; Nylin and Janz 2009; Janz 2011), and diversification rate heterogeneity (Janz et al 2001; Janz et al. 2006). Among the stated predictions of the OH, we were especially drawn to "This is an important part of our hypothesis for how plant diversity generates diversity in the insects that feed on them, but a final, and fundamental, part remains: does this diversification of the interaction indeed lead to elevated speciation rates?" (Janz and Nylin 2008). Previous work has investigated the association between host breadth and species richness. However, association between host breadth and diversification rates has not been explicitly examined. Speciation rate and species richness can be decoupled (Rabosky et al. 2012) or even negatively correlated (Stadler et al. 2014), and thus a correlation between species richness and host breadth (Janz et al. 2006) does not necessarily indicate a different rate of speciation. We asked if diversification rate variation in Nymphalidae is correlated with host breadth. Using a variety of tests, we found no evidence that greater host breadth is associated with increased diversification rate in the Nymphalidae.

The criticism leveled by Janz et al. (this issue) has focused on one particular analytical approach, our use of binary and multistate speciation and extinction models (hereafter, BiSSE, and MuSSE) (Maddison et al. 2007). We note that this criticism ignores the multitude of other approaches we employed and assume they take no issue with them; these approaches included: three independent approaches to identify diversification rate heterogeneity in the tree; examining host plant turnover between clades identified by the previous tests; and four independent methods to examine phylogenetic signal for host breadth (Hamm and Fordyce 2015). Finally, we used the BiSSE and MuSSE models to estimate diversification rates while explicitly considering host breadth. We had no agenda—and given the rigor of our study—we would have been satisfied with the answer regardless of the conclusion. However, based on all these analyses, we found no evidence of a positive association between host breadth and diversification rate.

The Right Model and Model Adequacy

Janz et al. (this issue) suggest that the BiSSE model does not properly describe the Oscillation Hypothesis. According to Janz et al. (this issue), the ClaSSE (Cladogenetic State change Speciation and Extinction model) model is more appropriate because it allows for both anagenetic (within a lineage) and cladogenetic (at a branching event) state changes, while BiSSE only allows the former state changes (Goldberg and Igic 2012). To further support the contention that BiSSE is not an appropriate model, Janz et al. note the work of Rabosky and Goldberg (2015), which correctly points out that when a phylogeny evolved under a process independent of the measured trait that BiSSE routinely reports high support for trait-dependent diversification. This is a legitimate concern because SSE models, including ClaSSE, assume that all diversification rate heterogeneity is attributed to only the character states included in the analysis.

Janz et al. (this issue) proposed that the "right" model is the ClaSSE, performed this analysis, and generated results that they interpreted as consistent with the predictions of the OH. Here, we demonstrate that the ClaSSE model and parameter estimates generated by Janz et al. (this issue) fit the data as well as a BiSSE model does. However, simulations using the parameter estimates from either model fail to predict the empirical data. We then follow the suggestion of Rabosky and Goldberg (2015) to explore the effects of hidden states on diversification rates in the Nymphalidae. To investigate the claims made by Janz et al. (this issue) regarding model adequacy, we conducted a series of tests to examine how well their modeling approach predicts the observed data. Specifically, we asked whether ClaSSE is a superior modeling approach for our data because it allows both anagenetic and cladogenetic state changes, and whether hidden states might have an important effect on diversification rates.

CLASSE

Let us assume for a moment that ClaSSE is the more appropriate model and the parameters estimated from this model properly represent what has occurred in the Nymphalidae. It follows that simulations generated under this framework (using the parameter estimates from Janz et al. [this issue]) should generate tips states distributed such that they produce phylogenetic signal similar to that which was observed in the data. These components are part of the model adequacy paradigm as promoted by O'Meara (2012), Beaulieu et al. (2013), and Pennell et al. (2015).

To assess phylogenetic signal we employed the metric K, which is measured on the interval $(0, \infty)$ and represents the scaled ratio of the observed mean square error (MSE) over the expected MSE under Brownian motion (Blomberg et al. 2003).

Under pure Brownian motion, the data will exhibit a high level statistical dependence (closely related taxa will resemble each other) and K will be 1.0. Conversely, when K is near 0.0, closely related taxa will not resemble each other. The K value observed in Hamm and Fordyce (2015) for monophagous specialists was 0.48 ($P = 0.0001$), indicating a moderate amount of phylogenetic signal. Using the parameters from Janz et al.'s (this issue) ClaSSE analysis, we simulated 10,000 phylogenetic trees (with the associated character states), and estimated K for each tree using 1000 simulations of a randomization test using tools in the diversitree (Fitzjohn 1212) and phytools packages (Revell 2012) in R.

We found that the Janz et al. ClaSSE model was not adequate to explain the observed data because it did not generate a distribution of tip states that matches the observed data. The Janz et al. (this issue) simulations generated a median K of 0.14 (95% HDI 0.076–0.21), far below the observed K value of 0.48 (Fig. 1). All code and data required to reproduce these analyses are freely available at DataDryad dx.doi.org/10.5061/dryad.3c7jb.

BISSE/HISSE

Our next point relates to the appropriateness of BiSSE as a test of the Oscillation Hypothesis. We reiterate that BiSSE was only one part of our article, and the conclusions of that work do not rest solely on the results from that analysis. As with ClaSSE, the BiSSE model returned levels of phylogenetic signal (nearly identical, actually) far below the observed level (Fig. 1). It appears that both BiSSE and ClaSSE models are not adequate to explain the role of host breadth on diversification in the Nymphalidae.

Recently, there has been significant progress in developing phylogenetic comparative methods that allow for the inclusion of hidden states that affect diversification. Published after the submission of our first article, the work of Beaulieu and O'Meara (2016) has produced the HiSSE (Hidden State Speciation and Extinction) model to address the concerns of Rabosky and Goldberg (2015). The HiSSE model allows speciation and extinction rate estimates to be made for both observed and hidden states, while also allowing transition rates to vary among observed and hidden states. For example, imagine that monophagous and polyphagous states were correlated with low and high adult dispersal, and high dispersal was strongly associated with higher diversification rates. If dispersal was not measured, one would be misled into thinking that diet breadth was a strong correlate of diversification. This is where the hidden state model comes in; HiSSE no longer requires the analysis to depend on a focal trait alone, but can instead estimate the importance of the trait as a component of overall diversification.

We fit a series of HiSSE models, including a model in which rates for character states evolve independently (a null model), a full model allowing independent rate estimates for all
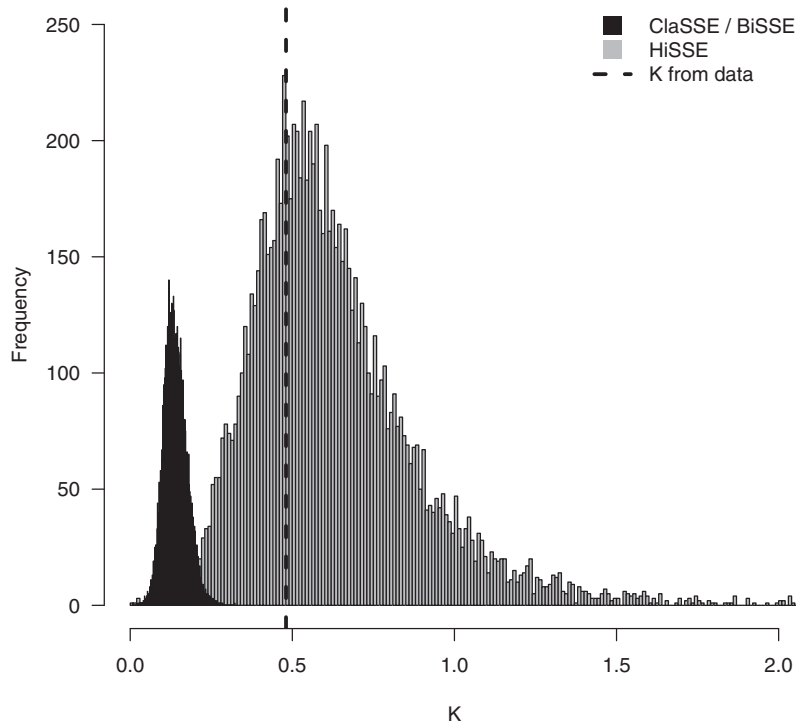
Figure 1. Phylogenetic signal (Blomberg's *K*) estimates based on 10,000 simulated trees for BiSSE, ClaSSE, and HiSSE models using the parameter estimates reported from, respectively: Hamm and Fordyce (2015), Janz et al. (2015), and Fordyce et al. (this article). The *K* value (0.48) from the empirical data is represented by a dashed vertical line. BiSSE and ClaSSE estimates overlapped entirely.

parameters, a model that allowed hidden effects to influence only monophagous lineages (feeding on one host plant family), and a model that allowed only hidden effect to influence polyphagous lineages (lineages that utilized more than one host plant). Our null HiSSE model (sensu Beaulieu and O'Meara in press) is similar to the BiSSE in number of parameters, but allows diversification and character state parameters to be entirely independent of one another.

We compared all HiSSE models, the Janz et al. (this issue) ClaSSE model, and the BiSSE model using AIC and AIC model weights (Akaike 1974; Burnham and Anderson 2002). The full model HiSSE was best by over 25 AIC points compared to other HiSSE models, better than ClaSSE/BiSSE by over 115 AIC points, and contained 99.99% of the AIC model weight (Table 1). The full HiSSE model indicates that lineages associated with monophagy have higher diversification rates compared to polyphagous lineages (Table 2), consistent with the conclusions of Hardy and Otto 2014 and Hamm and Fordyce 2015 and contrary to the conclusions of Janz et al. (this issue). However, the model also indicates that hidden factors have a greater influence of diversification rates in the Nymphalidae, suggesting that host breadth might not be the driver it was once considered. Following the protocol above, we simulated data under the HiSSE framework and conclude the full HiSSE model does a superior job of describing the data. Based on simulations, the full HiSSE model

Table 1. AIC scores, differences, and weights by model for analyses with monophagous specialists.

Model	AIC	ΔAIC	AIC weight
HiSSE—Full	3604.6	0.0	0.9999
HiSSE—Mono	3631.7	27.06	1.3×10^{-06}
HiSSE—Null	3659.7	55.09	1.1×10^{-12}
HiSSE—Poly	3714.4	109.8	1.4×10^{-24}
BiSSE	3719.7	115.1	9.9×10^{-26}
ClaSSE	3720.2	115.6	8.0×10^{-26}

generated a distribution of *K* values that were consistent with the observed data, $K = 0.55$ (95% HDI: 0.21–1.3) (Fig. 1).

In addition to the role of hidden states, one could also be concerned by the effect of phylogenetic pseudoreplication on our analyses (Maddison and Fitzjohn 2015). Phylogenetic pseudoreplication occurs when adjacent branches on a phylogenetic tree are treated as independent, but in reality are not independent due to shared common ancestry (Maddison and Fitzjohn 2015). One proposed method to address these concerns is the STructured RAte Permutation on Phylogenies (STRAPP) approach as proposed by Rabosky and Huang (2016). The STRAPP procedure assesses the correlation between character state and diversification rate in three steps: (1) diversification rates are estimated across the phylogeny, (2) an empirical test statistic between

Table 2. Speciation (λ), extinction (μ), and net diversification rate ($\theta = \lambda - \mu$) from full HiSSE model for monophagous specialists by state.

		λ	μ	θ	θ (total)
State 0	Observed	1.19×10^{-33}	2.05×10^{-33}	8.6×10^{-34}	0.096
	Hidden	0.096	7.6×10^{-18}	0.096	
State 1	Observed	3.85×10^{-3}	1.79×10^{-18}	3.85×10^{-3}	0.068
	Hidden	0.065	2.5×10^{-19}	0.065	

State 0 represents monophagous specialists, State 1 represents generalists.

diversification rates and character states is generated, and (3) the observed test statistic is compared to a null distribution generated by structured permutations (Rabosky and Huang 2016). Using the Nymphalidae dataset from Hamm and Fordyce (2015), we failed to find evidence of a correlation between diversification rate and host breadth ($P = 0.415$ [10,000 permutations, two-tailed Mann–Whitney U test]).

Conclusion

We stand by our original conclusion that, based on the data, we find no compelling evidence that greater host breadth is associated with increased diversification rates for the Nymphalidae. Clearly, the question to whether and how diversification rates and host breadth covary among herbivorous insects will remain an active area of research. To move forward, we propose several questions that we see as fundamental to increase our understanding of host breadth evolution for all herbivorous insects and, ultimately, how that might affect diversification rates. The list is not exhaustive and might have overlap with aspects of the OH as described by Janz. et al. (this issue). (1) What determines host breadth and what mechanism(s) facilitate host expansion? For example, is greater host breadth associated with a richer arsenal of counter-defenses in caterpillars against plant defenses, or simply counter-defenses that are more broadly effective? Comparative genomics and transcriptomics coupled with careful experimentation might provide fruitful avenues to answer this question. (2) If our HiSSE model is accurate, what are the hidden factors that are influencing diversification rate? Are other groups of herbivorous insects similarly affected by hidden, non-host breadth traits? Has enthusiasm for the role of host switches and the contraction/expansion of diet breadth caused us to overlook other important (possibly more important) aspects of herbivore biology? (3) Can we predict novel host associations based on current patterns of host use and reconstructed ancestral hosts. How robust are these predictions across the nymphalid tree?

Finally, we agree that "the fluttering hypothesis" might sound silly—that is why we never really proposed it. We do believe, however, that a hypothesis as mature as the OH is ripe for an explicit mathematical treatment. Testing hypotheses based on a thought

model can only take us so far and easily lead to misunderstandings. It is much easier for a productive dialogue to emerge when centered on an explicit model. If the OH is an important idea to be considered, it deserves such treatment.

DATA ARCHIVING

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