MTH note: This document is the (very slightly) edited project description of the proposal that was funded by NSF. The proposal was written in the summer of 2012 by Mark T. Holder, Bruce S. Lieberman, and Tracy A. Heath. The position of the edits is shown in blue text.

Integrating Fossil Data into Likelihood-based Phylogenetic Analyses with Trilobites as a Model System

1 Objectives

The primary objective of the proposed research is to develop improved methods for likelihood-based phylogenetic analysis of morphological data with an emphasis on fossil taxa. Many analyses of molecular data use likelihood-based approaches, so the current lack of compelling models of character evolution for morphology is a serious impediment to integrating paleontological and neontological data. We will develop models and software that can estimate phylogenies from character data. We will use examples from the Trilobita to test our methods. Trilobita is an exceptionally diverse fossil clade that has been the subject of numerous phylogenetic analyses at several taxonomic scales (Lieberman and Karim, 2010a). Our new models of character change will be implemented in software that will estimate divergence times, and speciation and extinction rates. The results of this grant will dramatically improve researchers' ability to put a time scale on the Tree of Life and explore evolutionary questions about the tempo of character changes and macroevolutionary processes. The proposal is structured into the following sections:

Section 2 Background: a discussion of prior work and preliminary data,

Section 3 Research questions and approaches: a presentation of five specific goals to be addressed and a discussion of the approaches to be taken,

Section 4 Software: the details of the software to be written to enable the research,

Section 5 Broader Impacts: a summary of the broader impacts of the proposed work, and

Section 6 Timeline: a schedule for accomplishing the work including a discussion of the role of each researcher.

2 Background

2.1 Phylogenetic estimates for fossil taxa – Parsimony has been widely applied to phylogenetic studies of extant and fossil taxa. Previous maximum likelihood (ML) approaches for fossil data specifically focused on using simulations to assess the degree to which parsimony-based phylogenies are consistent with stratigraphic data (Wagner, 1995, 1998). Such approaches have proven efficacious in a variety of contexts (e.g., Wagner, 2000), but our aim is different. Instead, we will adapt ML models similar to those used in molecular phylogenetics (e.g. Neyman, 1971; Felsenstein, 1981) for use with discrete morphological characters.

Analyses with many fossil taxa often show poor resolution because these taxa typically lack data from soft tissues. This leads to sparse matrices with a great deal of missing data. Supertree approaches (e.g. Sanderson, Purvis and Henze, 1998; Bininda-Emonds, Gittleman and Steel, 2002; Wilkinson et al., 2005) have frequently been used when overlap in characters across all taxa is poor. These methods estimate trees for small, overlapping sets of taxa, and then combine these estimates to generate a higher-level phylogeny. Supertree approaches are tenable when applied to fossil taxa (see Lieberman, 2002). However, the ability to analyze character matrices of morphological and paleontological data would provide a more powerful method for resolving conflicting signals, and would enable a more sophisticated approach to time-calibrating phylogenies (e.g. Pyron, 2011; Ronquist et al., 2012). The models that we propose to develop will enable such analyses.

The complicated nature of character choice and character state delimitation in morphological systematics presents a challenge to applying simple Markov models to these data. Lewis (2001) pointed out the need to

condition likelihood calculations on the fact that morphological datasets include only variable data patterns. He introduced the M*k*v model to deal with such data. Many morphological character matrices only contain "parsimony-informative" characters (characters in which the parsimony score differs across different trees). Allman, Holder and Rhodes (2010) showed that ignoring the fact that data have been filtered to exclude parsimony-uninformative sites can result in statistically inconsistent tree inference; they also proved that it is possible to correctly estimate large trees if the likelihood calculations account for this data filtering.

The exclusion of constant or parsimony-uninformative characters represents a form of ascertainment bias. Lewis' (2001) correction for the lack of constant patterns treats the process of scoring morphological characters as a process of randomly selecting a character from a universe of possible characters and rejecting any constant character. Similar techniques of accounting for the lack of parsimony-uninformative patterns (Nylander et al., 2004; Allman, Holder and Rhodes, 2010) also view matrix composition as filtered random sampling. These approaches fail to recognize other important aspects of morphological data collection. In particular, morphological systematists are extraordinarily adept at detecting potentially valuable characters. Therefore, the character matrix that serves as the basis for tree inference should not be treated as a collection of random draws of traits. If there is a macroscopic trait that varies slowly across a taxonomic group, there is a very good chance that the character will be included in the data matrix. Accommodating the ability of systematists to "scan" the holomorphology of a specimen and score the most promising phylogenetic changes can be very crucial to evaluating the probability that two specimens belong to the same species. Recently Rosenblum et al. (2012) emphasized the importance of considering "ephemeral species" in models of diversification. Fitting such models to fossil data will require a model of the evolution of diagnosability in a new species.

n addition to ascertainment biases, the lack of independence between different characters can plague systematics. Methods such as the "threshold" approach (Felsenstein, 2005) and models of correlated change among discrete characters (Pagel, 1994; Pagel, Meade and Barker, 2004; Pagel and Meade, 2006) represent potential solutions to dependence among characters. However, these approaches require that the suite of potentially interdependent characters be identified *a priori*. The methods can also lead to dramatically slower analyses if the number of interacting characters is large. Markov chain Monte Carlo (MCMC) techniques for relaxing the character-independence assumption have been developed for the evolution of protein-coding sequences (Robinson et al., 2003; Rodrigue et al., 2005; Rodrigue, Philippe and Lartillot, 2006). These methods explore the state space of all possible character histories across a tree. They use biophysical models to predict fitness of sequences to avoid overparameterization. We lack general models for predicting the fitness of different combinations of morphological character states, but we can rely on another well-developed statistical toolkit for avoiding overparameterization. Dirichlet process models (Ferguson, 1973; Antoniak, 1974) provide a means of exploring a continuum of models from a homogeneous process with few parameters to a rich description of very heterogeneous data. They have been applied to a number of problems in phylogenetics (Lartillot and Phillipe, 2004; Ane et al., 2007; Huelsenbeck et al., 2006; Huelsenbeck and Suchard, 2007; Heath, Holder and Huelsenbeck, 2012; Heath, 2012), but they have yet to be applied to models of morphological characters.

2.2 Estimating speciation and extinction rates — The development and use of methods to analyze speciation and extinction rates has been an important area of research in both paleontological (e.g. Stanley, 1979; Raup, 1985; Foote, 2000; Lieberman, 2001c; Rode and Lieberman, 2005a; Abe and Lieberman, 2009; Etienne and Apol, 2011) and neontological applications (e.g. Nee, Mooers and Harvey, 1992; Sanderson and Donoghue, 1996; Paradis, 1997; Heard and Mooers, 2000; Nee, 2001, 2006; Rabosky, 2006; Maddison, Midford and Otto, 2007; FitzJohn, Maddison and Otto, 2009; Alfaro et al., 2009; Cowman and Bellwood, 2011; Silvestro, Schnitzler and Zizka, 2011). Although several software packages can be used to

calculate speciation and extinction rates for extant taxa using molecular phylogenetic data such as Geiger (Harmon et al., 2008) and MEDUSA (Alfaro et al., 2009), equivalent applications are not widely available for the analysis of fossil phylogenies. Previous approaches to analyzing speciation and extinction rates in fossil taxa thus typically required manual calculations. This is straightforward under simple deterministic methods (e.g., Stanley, 1979; Foote, 2000; Rode and Lieberman, 2005a; Abe and Lieberman, 2009). However, it is analytically more complicated when probabilistic approaches are employed (see Lieberman, 2001c), necessitating the use of programs like Maple (Waterloo, 2011) or MatLab (MathWorks, 2011); also see Etienne and Apol (2011). Discrete time approaches have also been developed for analysis of fossil taxa, (e.g., the Bienaymé-Galton-Watson branching process, see Gilinsky and Good, 1991), but since some of the assumptions needed to implement this model may not be realistic (Sanderson and Donoghue, 1996; Lieberman, 2001c) we will not focus on these here.

When estimating speciation and extinction rates from the fossil record, the data used are diversity estimates and information about the stratigraphic distribution of species. To the extent that phylogenetic information has been incorporated in the analysis of diversification rates in fossil taxa (e.g. Lieberman, 2001c; Rode and Lieberman, 2005b; Abe and Lieberman, 2009; Stigall, 2010), it is only done to augment diversity estimates in combination with stratigraphy, in a ghost lineage approach. Branch length information, in conjunction with phylogenetic topologies of fossil taxa, has not been fully taken into account in analyses of diversification rate. This is in stark contrast to speciation and extinction rate analyses of molecular phylogenies as these explicitly utilize branch length information (based on sequence characters) in their rate calculations (e.g. Harmon et al., 2008; Alfaro et al., 2009). Recently Brown, FitzJohn, Alfaro, and Harmon have extended the MEDUSA approach for estimating patterns of diversification over trees to include some information from data on the abundance of taxonomic groups at particular time horizons (unpublished, LH pers. comm.). However their approach does not deal with the specific placement of fossil taxa. Thus, their method does not account for phylogenetic uncertainty or use detailed information about the temporal extent of fossil taxa (LH, pers. comm.).

The current state of the art for fossil phylogenies not only represents a serious handicap to producing robust speciation and extinction rate calculations, it also represents a significant impediment to integrating diversification rate analyses of extant and fossil taxa. With the methods we propose to develop we aim to rectify this situation. This will place diversification rate analyses of fossil lineages on the same statistical footing as those of extant taxa considered by molecular phylogenies. This is a particularly appealing goal because fossil data clearly contain much more information about extinction than the information that can be gleaned from neontological data alone.

By considering morphological character information on phylogenies in conjunction with stratigraphy to infer branch lengths and using this information to derive speciation and extinction rate estimates, we will improve on diversification rate estimates that use stratigraphic distributions of species alone. While stratigraphy provides only a single line of evidence, it is a very valuable and under-utilized source of information about time scale across a phylogeny. The more characters used to derive branch length estimates, generally the more robust those estimates are expected to be. Although stratigraphic information is very important, it does not always provide maximal information or resolution about branch length in any given phylogeny. This is because in analyses of diversification rate for fossil taxa it is necessary to consider the occurrence of species relative to single stratigraphic bins that possess bracketing radiometric dates. Available radiometric dates may be scanty, such that many species must be assigned to the same bin and assumed to have the same point of origin. Depending on phylogenetic topology, this could mean that many species would be assigned the same branch length. Incorporating additional morphological information into branch length estimation could partly ameliorate this problem and better resolve differences in branch length between stratigraphi-

cally co-distributed, closely related species. This in turn should be expected to produce more accurate and refined diversification rate estimates.

2.3 Trilobite phylogenetics — Our analyses will focus on two quite distinct groups of trilobites, the Olenellina (Figure 1a) and the Cheiruridae. Because of their morphological complexity, diversity, dense fossil record, and important role in pre-existing phylogenetic studies, these trilobite groups are excellent candidates for the development of likelihood-based phylogenetic analyses and speciation and extinction rate studies. The Olenellina comprises more than 125 species, the Cheiruridae, more than 300 species. Furthermore, they belong to different orders with very different evolutionary histories (Fortey, 2001), which

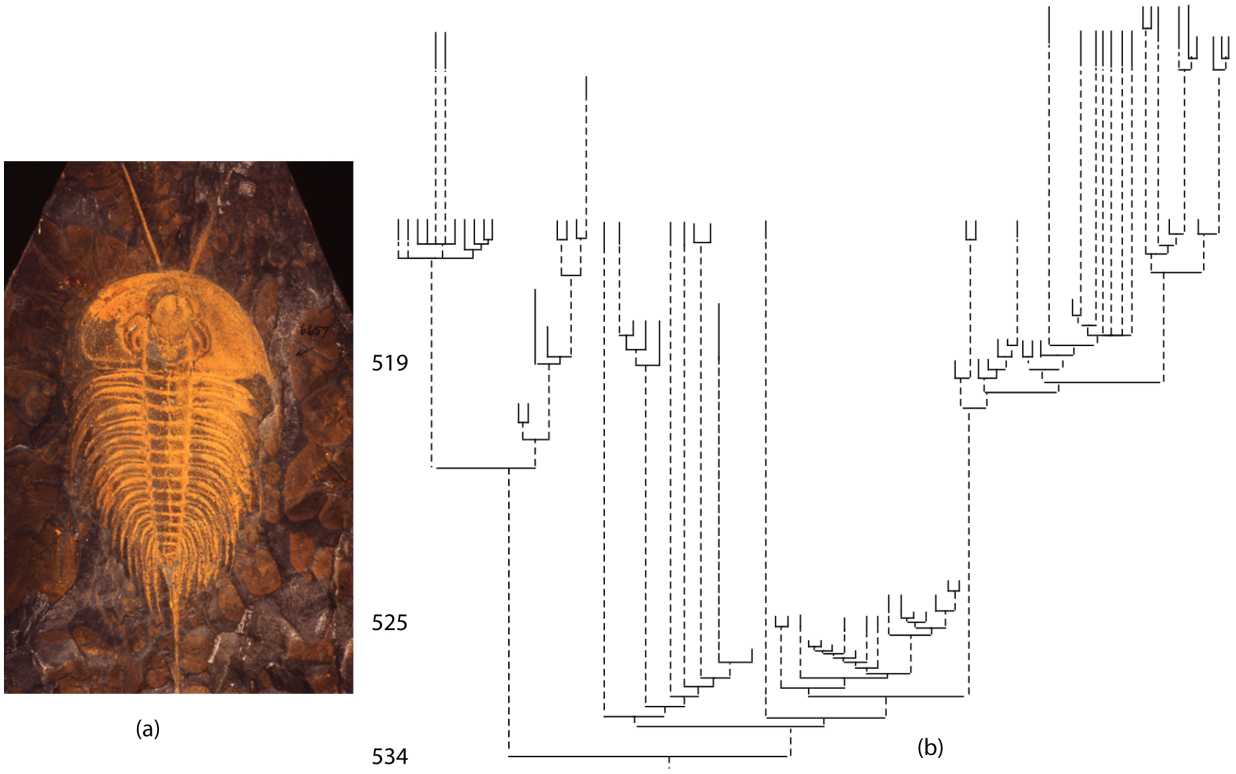

Figure 1: (a) The holotype of *Olenellus getzi* Dunbar 1925. (b) A phylogeny of the Olenelloidea from Lieberman (1999) with time scale shown in millions of years before present. Dashed lines represent ghost lineages.

can potentially expand our understanding of character variation in the group and aid in developing effective character models. For instance, the Olenellina are exclusively Early Cambrian, ∼525-510 million years ago (Ma), and diversified during the Cambrian radiation interval (Lieberman and Karim, 2010b). They constitute the sister group to all other eutrilobites and are plesiomorphic in their possession of, among other characters, a diminutive pygidium and absent dorsal facial sutures on the cephalon (Lieberman, 1998, 2002). They have figured prominently in studies of evolutionary and biogeographic patterns during the Cambrian radiation (Lieberman, 2001a, 2003; Meert and Lieberman, 2004, 2008). By contrast, the Cheiruridae span the Ordovician-Devonian, ∼470-380Ma and belong to the derived Order Phacopida (Chatterton et al., 1990; Fortey, 2001). They have been used to study evolutionary patterns during the Ordovician radiation and the end Ordovician mass extinction (Adrain, Fortey and Westrop, 1998; Congreve and Lieberman, 2011a;

Congreve, 2012). Considering both a Cambrian and a post-Cambrian group is worthwhile, as there may be some differences in patterns of character variation in Cambrian relative to post-Cambrian arthropod groups in general, and trilobite groups in particular (see discussion in Hughes, 1991; Hughes, Chapman and Adrain, 1999; Smith and Lieberman, 1999; Briggs and Fortey, 2005).

Each group has been the subject of many parsimony based phylogenetic analyses by BSL and colleagues; so numerous datasets are available for the Olenellina (Lieberman, 1998, 1999, 2001c, 2002; Gapp et al., 2011) and the Cheiruridae (Adrain, 1998; Congreve and Lieberman, 2010, 2011c,b; Gapp, Holder and Lieberman, 2011a, and Congreve (in prep.)). For Olenellina, a detailed species-level phylogeny exists, developed using parsimony and supertree methods (Lieberman, 2002). We present a preliminary analysis of one subgroup, the Olenelloidea, below.

Phylogenies exist for more than 130 species in Cheiruridae. These trees span several different subfamilies, and they can be combined into a single supertree. In all cases these trilobite "species" are diagnosably distinct morphologically and may be equivalent to phylogenetic species as they are diagnosed by autapomorphies and a unique combination of primitive and derived characters. The characters used to develop these phylogenetic hypotheses are from the mineralized exoskeleton, as the internal anatomy of trilobites is only rarely preserved in the fossil record. Characters include both qualitative and quantitative (but not continuous) features of the external anatomy (for additional examples see Fortey and Chatterton, 1988; Edgecombe, 1992; Lieberman, 1994; Adrain, 1998, 2003; Chatterton et al., 1998; Ebach and McNamara, 2002; Amati and Westrop, 2004; Pollitt, Fortey and Wills, 2005; Adrain and Westrop, 2007) and emphasize the adult, holaspid phase of the trilobite exoskeleton. Ontogenetic information and juvenile stages are not available for the bulk of the species considered by these analyses.

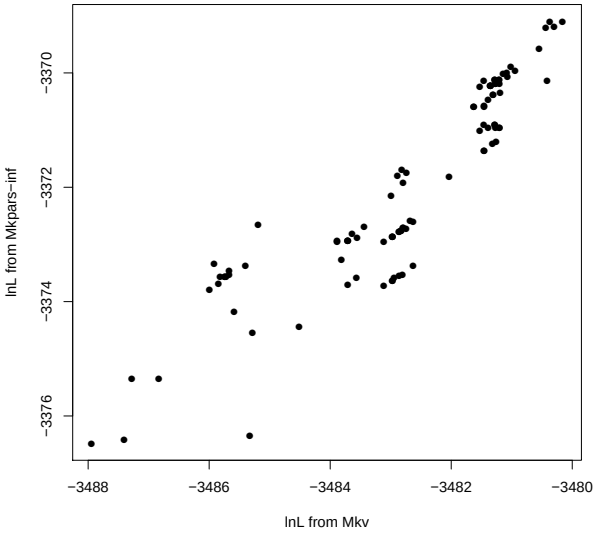

Figure 2: Scatterplot of the log-likelihood of 100 most parsimonious trees under the M*k*v model (x-axis) and M*k*-parsimony-informative model (y-axis).

2.4 Preliminary data — As a test case, we applied ML methods for correcting for ascertainment bias to a supermatrix generated for a group of 81 species of Early Cambrian trilobites comprising the Olenelloidea, a diverse, morphologically complex clade. A detailed phylogenetic picture of this group was obtained through the use of several parsimony analyses and a supertree approach (Lieberman, 1998, 1999, 2002). Lieberman (1998) first conducted a higher level phylogenetic analysis for this group. A series of subclade analyses were conducted by Lieberman (1999), and these were stitched together to form a supertree (Lieberman, 1999, 2002, see Figure 1b). In our new approach, we (Gapp, Holder and Lieberman, 2011b) developed and coded 88 morphological characters for all 81 olenelloid species in a single analysis. When we subjected the new 81-taxon data matrix to parsimony analysis numerous trees resulted, with the strict consensus being a complete polytomy. This situation arose because many species were known from incomplete material, and their position varied across most parsimonious trees. In the supertree approach (Lieberman, 2002), it was possible to fit incomplete material into various clades based on the presence of certain distinctive synapomorphies and

then the smaller scale phylogenetic analyses could retrieve resolution.

We also analyzed the dataset using ML (Gapp, Holder and Lieberman, 2011b). Version 2.0 of GARLI (Zwickl, 2006) supports searching under the M*k*v model (Lewis, 2001). We extended this software to allow for the analysis of datasets that contain only parsimony-informative characters. We have not yet implemented the dynamic programming techniques required to efficiently calculate the likelihood for the Mk-parsimony-informative-only (M*k*pars-inf) model (Nylander et al., 2004; Allman, Holder and Rhodes, 2010). Thus, our preliminary implementation did not allow us to conduct thorough searching or bootstrapping. Nevertheless, we can see (Fig 2) that the ML scoring of 100 different most-parsimonious trees under the M*k*v and the M*k*pars-inf model would give different rankings of trees. This demonstrates that using the correct form of conditioning in the ML framework can influence tree choice for fossil data. The trees estimated under ML retrieved some clades also found by the supertree approach, but some differences were also found. These principally involve questionable placement of incompletely known taxa.

3 Research Questions and Approach

The specific aims of this research are to:

1. Develop algorithms for dealing with character-filtering ascertainment bias for multi-state characters, and implement these methods in high performance ML tree searching software.
2. Develop models for the evolution of species diagnosability.
3. Integrate these models of morphological character evolution and stratigraphic information into Bayesian MCMC software for divergence time estimation.
4. Implement several macroevolutionary models of diversification in the same Bayesian software to enable various macroevolutionary analyses, including the inference of changes of diversification rates.
5. Implement MCMC methods for inferring trees from co-evolving suites of morphological characters, using nonparametric Bayesian methods.

Each of these methodological developments will be tested using simulations and cross-validation approaches applied to the rich data available for trilobites in the groups Cheiruridae and Olenellina. We will expand on our preliminary data generated for Olenellodea to produce a single phylogenetic matrix to encompass all of Olenellina. We will combine the phylogenetic perspective that currently exists for the species in Cheiruridae into a single analysis. This will also allow us to compare in detail the differences that result between paleontological analyses that use parsimony and those that use ML.

Goal 1: Models for ascertainment biases — -

MTH note: (slight editing was done in this section to redact undergraduate's name) As mentioned above, MTH added a prototype implementation of the M*k*pars-inf model to GARLI. During the Spring of 2012, MTH has worked with an undergraduate student majoring in math who was recruited via KU's Initiative for Maximizing Student Diversity (IMSD). They are developing the dynamic programming methods needed to efficiently enable searching under M*k*pars-inf when there more than three states. We will add these methods to GARLI during the summer of 2013 while more rigorously testing the behavior of the methods. Because this form of ascertainment bias correction views matrix construction as merely filtering of randomly selected characters, it will be trivial to adopt current data simulators (which generate random characters) to produce filtered matrices. Thorough testing of the model on trilobite datasets and via simulation will continue in the fall of 2013. The programming will be performed by MTH, with an undergraduate student assisting in running the simulations and summarizing their results. As part of this work, the undergraduate will be trained in the writing of software for biological analyses.

Goal 2: Modeling the evolution of species diagnosability — When multiple, morphologically indistinguishable specimens are recovered they can be assigned to the same species. However, this inference

is not guaranteed to be accurate. Using explicit models of speciation and extinction (as we will do in goals 3 and 4), will induce a probability distribution on competing hypotheses about whether or not two specimens belong to the same species (Felsenstein, 2012). Such models can make probability statements about the possibility that a systematist has incorrectly clumped two specimens from different species into a single species. However, if these statistical models treat the characters in the matrix as random draws from a universe of characters (with subsequent filtering as discussed above), there will be a tendency for the statistical analysis to split specimens from a single evolving lineage into too many species. This will occur because the model of character evolution will not interpret an identical array of characters states as very compelling evidence of conspecificity. After all, morphological data matrices typically contain a relatively small number of characters. This treatment would underestimate the judgement of a systematist about the species status of specimens. Part of this deficiency can be corrected by formally expressing the fact that a morphologist can efficiently scan the morphology of a specimen and choose diagnosable traits that improve species delimitation. Thus, the observation that two specimens show the same array of character states indicates not only that they are similar to each other at M randomly selected characters (where M is the number of characters in the matrix), but also that a trained systematist has considered a potentially vast number of possible characters and has found none that merit designation of multiple species. Following a suggestion of Felsenstein (pers. comm.), we will consider the event "no diagnosable characters have evolved in a universe of X potential characters" (where X is unknown, but $X > M$) as an event that should be included in the calculation of the likelihood. This will allow us to consider the extent to which our trilobite data suggest a discrepancy between inferred and actual amount of species diversity. While this goal is not a major component of the grant in scope (it will not entail dramatically different likelihood calculations), it will be a necessary step for subsequent goals.

Goal 3: Integration of fossil data and stratigraphy into Bayesian divergence time estimation
While much progress has been made on relaxing the molecular clock assumptions commonly used in divergence time estimation (e.g. Heath, Holder and Huelsenbeck, 2012), placing fossil taxa into their correct phylogenetic context remains a crucial and difficult endeavor (Parham and Irmis, 2008). Addressing this weakness in studies of the timing of evolutionary events will require improvements on using morphological characters – precisely the type of modeling effort that we propose to conduct. While the models used to accomplish this goal (and goals 4 and 5) could be implemented in a Bayesian or ML framework, we will pursue Bayesian approaches because our techniques rely on inference under parameter rich models. Integrating over uncertainty about the values of these parameters is relatively easy in a Bayesian MCMC analysis and will avoid error introduced by using the ML estimate for many nuisance parameters.

In addition to accounting for the way morphological data are collected, it is also critical that we develop models that consider how rates of morphological evolution vary across the tree of life. It is unlikely that the rate of morphological evolution is constant over time (i.e. we do not expect to see a "morphological clock"), furthermore, it is not clear that the rates of change conform to the standard relaxed clock models often applied to molecular phylogenies (Huelsenbeck, Larget and Swofford, 2000; Kishino, Thorne and Bruno, 2001; Drummond et al., 2006; Lepage et al., 2006; Rannala and Yang, 2007; Drummond and Suchard, 2010; Heath, Holder and Huelsenbeck, 2012). Thus, a model for the rate of character change among lineages will be an important component of modeling morphological evolution. In recent work (Heath, Holder and Huelsenbeck, 2012) we introduced a flexible model of among-lineage substitution rate variation for Bayesian divergence time estimation using molecular data. Our method applies a Dirichlet process mixture model as a prior on among-lineage rate variation. This model assumes that lineages fall within distinct rate-parameter classes and can flexibly accommodate a range of rate variation models including a strict clock, local clocks, and independent rates models. When applied to molecular data simulated under different models for lineage-

specific substitution rate variation, we showed that assuming a Dirichlet process model results in robust and accurate estimates of branch rates and node ages (Heath, Holder and Huelsenbeck, 2012). Because this relaxed clock model is a descriptive one, it can also be applied to analyses of morphological data. Conveniently, the nature of the Dirichlet process model will allow for the comparison of different rate-variation models using Bayes factors (Kass and Raftery, 1995). Using this framework, it will be possible to statistically test the support for different models of morphological rate variation in diverse lineages of fossil taxa. Furthermore, because the Dirichlet process prior can account for latent partitioning structure in branch rates, Bayesian inference under this model can identify lineages that evolve at the same rate (Heath, Holder and Huelsenbeck, 2012).

Recent pioneering work by Pyron (2011) and Ronquist et al. (2012) applied existing models of morphological evolution and substitution rate variation to "total evidence" datasets. These studies combine molecular data from extant taxa with matrices of discrete morphological characters coded for both extant and extinct specimens. With these data it is possible to simultaneously estimate the tree topology and divergence times using MCMC. These studies advanced the field of divergence time estimation by allowing for broader representation of information from the fossil record in analyses that seek to understand the timing of evolutionary events (Pyron, 2011; Ronquist et al., 2012). Additionally, these approaches provide a means for accounting for uncertainty in the placement of fossil taxa and eliminate the need for specifying prior distributions on internal nodes. We will build on this work by integrating improved models for among-lineage variation in rates of morphological change with new models for morphological evolution. This will enhance our ability to represent uncertainty in estimates of species divergence times and the tempo of evolution. However, building on these methodological improvements will also require developing advanced stochastic branching models that integrate processes of speciation and extinction with parameters accounting for rates of fossilization, preservation, and recovery of fossil lineages. Toward that end, we will pursue the objectives outlined in the following section (goal 4).

Goal 4: Full integration of fossil taxa into analyses of the rates of diversification Our inclusion of fossil data and stratigraphic information will allow us to formulate models in which rates of character change are affected by the number of speciation events and the age of a species. Thus, our methods will also enable novel approaches to test for, and study the implications of, punctuated equilibrium (Eldredge and Gould, 1972; Eldredge et al., 2005). In particular, several interesting studies have been conducted (Webster, Gaines and Hughes, 2008; Pagel, Venditti and Meade, 2006) using molecular sequence data to look at the relative amount of evolutionary change that tends to happen at speciation events or nodes on a phylogeny as opposed to the amount of morphological change that could be treated as phyletic divergence along the branches of a phylogeny. These methods included the ability to correct for the node-density artifact (Venditti, Meade and Pagel, 2006). Related approaches have even been applied to look at the evolution of languages (Atkinson et al., 2008). Currently it is possible to consider this issue with individual traits mapped across a phylogeny using the GEIGER (Harmon et al., 2008) software package (see Hunt, 2012), but this is a very different aim from our proposed approach. Instead, our approach will enable analysis of the complete morphological datasets available from fossil phylogenies to consider this issue. These different approaches are complementary, and access to different methods allows researchers to approach this issue in a variety of different ways and contexts, depending on available datasets.

We will investigate new forms of relaxed clock models for the morphological data from fossils. By assigning some probability of character change occurring instantaneously at speciation events, we can fit models of punctuated equilibrium. Other models for the changes in the tempo of the rates of character evolution will be implemented using transformations of the branch lengths, this will enable statistical tests of different macroevolutionary phenomena. For example, we will implement models that allow the rate

of morphological change to slow as the age of a species increases. Such models could be used to explore canalization of phenotype following speciation, as some have suggested this may be an important phenomena (e.g. Gould, 1980; Riedl, 1978). Using both of the large trilobite datasets we will explore evidence for canalization and punctuated equilibrium.

While the estimation of speciation and extinction rates could be conducted in ML using a penalized likelihood approach (cf. Sanderson, 2002), we will focus on Bayesian approaches because effective MCMC proposals (e.g. Neal, 2000; Silvestro, Schnitzler and Zizka, 2011; Heath, Holder and Huelsenbeck, 2012; Heath, 2012) enable efficient model selection during the estimation procedure. Stadler (2010) has derived the probability densities associated with different tree shapes under birth-death processes when tips of the tree are not contemporaneous. Her methods allow for the correct calculation of tree probabilities when species are sampled over time. We will implement the framework of Stadler (Stadler, 2010, 2011b) into software for Bayesian estimation of speciation and extinction rates using MCMC. We will also extend Stadler's method to allow for changes in the speciation and extinction rates by using reversible-jump MCMC methods (Green, 1995) to explore the space of models. With this framework in place, one could further extend the stochastic tree models to account for the factors that influence the processes of fossilization, preservation, and recovery that are important considerations for any analysis of fossil taxa. Disentangling speciation and extinction rates is extremely difficult from data based only on extant species (Rabosky, 2009; Simpson et al., 2011), but having a rich fossil record will often (though not always, see Morlon, Parsons and Plotkin, 2011) dramatically enhance the statistical power available for divergence time estimation and the inference of diversification rates (Wilkinson et al., 2011; Quental and Marshall, 2010; Morlon, Parsons and Plotkin, 2011; Etienne et al., 2012). The potential to add in fossil taxa of course greatly improves opportunities for sampling the diversity of extant clades with a fossil record, and it has been demonstrated that better sampling a clade's diversity improves diversification rate estimates (Cusimano and Renner, 2010; Brock, Harmon and Alfaro, 2011; Höhna et al., 2011). Recent advances in the calculations of Bayes factors (e.g. Xie et al., 2011) will allow us to test these models against each other using the trilobite data.

The computational machinery for implementing many of these models is straightforward. For example, models encapsulating some aspects of punctuated equilibrium and canalization can be implemented in software by a simple transformation of branch lengths. What has been difficult to do in the past has been exploring these models in ways that appropriately acknowledges phylogenetic uncertainty. Thus, our work on goals 1-3 will be key to effectively exploring these macroevolutionary models. Knowledge about the temporal range of species is crucial for addressing such questions, and the detailed data on Olenellina and the Cheiruridae will serve as an ideal test of our methods. We will recalculate speciation and extinction rates for the two trilobite clades using our newly developed perspective. Further, we will consider how these change previous understanding (Congreve and Lieberman, 2011a; Lieberman, 2001b, 2003) of evolutionary tempo in trilobites during the Cambrian radiation and the end Ordovician mass extinction, two key time periods in the history of life. Our ultimate goal is to unite the wealth of data from the rich fossil record of trilobites with some of the most powerful methodological tools that have been developed.

Our development of advanced, complex macroevolutionary models will be facilitated by collaborative work with Tanja Stadler. Dr. Stadler described a branching model used to infer step-wise shifts in rates of diversification, allowing for detection of mass extinction events (Stadler, 2011a). This model can be extended to account for break points at geologic strata and implemented in a Bayesian framework. Dr. Stadler is currently collaborating with TAH on adapting branching-process models to accommodate calibration information and her expertise will be a valuable contribution to our project.

We will conduct computer simulations to validate the inference methods using a tree and taphonomic process simulation program written by TAH. This software generates tree topologies under a range of birth-

death branching models using the generalized sampling approach (Stadler, 2011b). Fossilization events are generated according to a Poisson process along the tree, where the rate of fossilization can be varied over time or over the tree. Biases in preservation or recovery are modeled according to an autocorrelated Brownian motion model. Moreover, gaps in the rock record can be incorporated into the fossil recovery model. With these trees and fossils, we can simulate both molecular sequence data and morphological characters for fossil and extant lineages under complex models of lineage-specific rate variation. This simulator was designed to recover a set of fossils for divergence time analysis under realistic conditions. With these data in hand, we can evaluate the power and performance of methods for answering questions about the fossil record in a phylogenetic context.

Goal 5: Accommodating non-independence of characters Virtually all phylogenetic methods assume that different characters provide independent evidence of phylogenetic relationships. That assumption can be violated for many different types of data, but it can be difficult to detect dependence among characters in fossil (or extant) taxa. For instance, in the context of paleontological analyses, we rarely have detailed ontogenetic series or data about correlations in characters from polymorphic populations. However, we will develop methods to analyze the discrete character data by combining multiple characters into a single character with many states and then analyzing it as a character suite. This may provide a way around the difficulty of non-independent characters in paleontological (and neontological) phylogenetic datasets. The foundational work on this type "character suite model" which allows correlated evolution has already been done (e.g. Pagel, 1994; Pagel, Meade and Barker, 2004; Pagel and Meade, 2006).

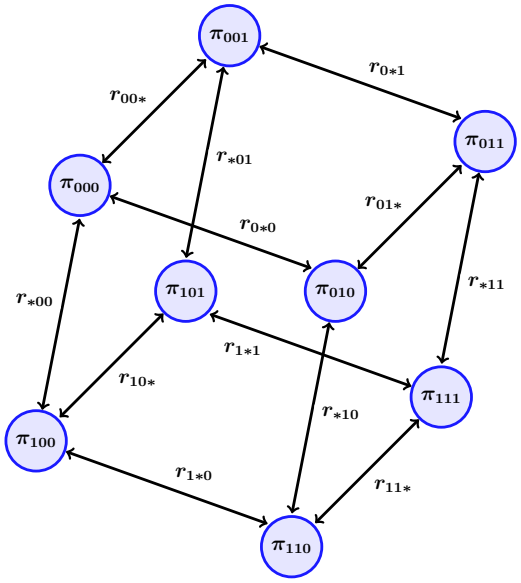

Figure 3: Depiction of a model for a character suite of three binary characters.

Figure 3 depicts an example of a character suite model created by uniting three characters (each of which had two states) into one character suite with eight states. The π parameters represent the state frequencies for the different character suite states (the subscript used for each state represents the binary state for each of the 3 original characters). The r parameters represent the exchangeability rate parameters (the subscript shows an $*$ for the original character which is changing state, and the other context of the two characters which are not changing). In the typical uses of these character suite models (e.g. the usage in Pagel, 1994), the biologist specifies the characters that may be undergoing correlated evolution *a priori*. While this can be convenient if we know which characters might be evolving in a dependent manner, we rarely have this detailed knowledge. We will use a model mixing approach to allow character suites to be identified by the patterns in the data. Dirichlet process priors (DPP) provide a nonparametric approach to partitioning a group of entities into subsets. The method uses a single parameter (the concentration parameter) to induce a probability statement over all possible partions. Neal (2000) has developed very generic, but efficient approaches for implementing multi-model inference using DPP.

We will use the DPP approach to explore models in which the grouping of sites into interdependent suites of characters is an unknown variable to be estimated. Thus, sites will be the entities modeled using the DPP, and placing two sites within the same group will cause them to be treated as a co-evolving suite (as

in Figure 3). In many applications of phylogenetics, the concentration parameter of the DPP is used to favor grouping many entities into as few groups as necessary. In our context, we will use a strong prior on the concentration parameter to prefer the partition of each character belonging to its own character suite. This partitioning corresponds to the assumption that all of the characters are independent (a standard assumption in phylogenetic analysis). Our DPP implementation will not rule out the possibility that characters can belong to the same suite, however. During the course of a MCMC run, new models of correlated evolution will be proposed. When this occurs, the characters that are grouped will be recoded (internally by the program conducting the MCMC) into a multistate suite (as in Figure 3). This approach will allow the phylogenetic analysis to be open to the possibility that characters are not evolving independently, but will tend to discount that possibility unless it leads to a substantially improved explanation of the data. With our trilobite data we can also explore the extent to which these character data do or do not show independence.

Two immediate issues arise when contemplating the model of character suites outlined above: how can we avoid introducing too many parameters in the model of character evolution, and how can the computation be accomplished if the state space of the character suites becomes very large? When recoding multiple characters into a single suite, the number of possible states grows exponentially with the number of included characters. In addition to requiring an equilibrium state frequency for every state (the π parameters in Figure 3), a full specification of the model will require the specification of exchangeability parameters (the r parameters in Figure 3). It is unlikely that the data will strongly prefer specific values for this vast number of parameters. We will focus our efforts on models in which all of the exchangeability parameters take the same value, but the state frequencies are estimated. Even in this case, the number of parameters can be huge. Once again we can employ a nonparametric DPP approach to learn about the number of distinct state frequency parameters that best explain the data. By treating the frequency parameters as the entities modeled by this DPP, we can favor models with few parameters, but be responsive to signal in the data that some combinations of character states are preferred (or avoided). To overcome the computational hurdles associated with applying Felsenstein's pruning algorithm on large state spaces, we can co-opt the MCMC machinery pioneered by data augmentation methods (Robinson et al., 2003; Rodrigue et al., 2005; Rodrigue, Philippe and Lartillot, 2006). At threshold values corresponding to a computationally expensive numbers of states, our MCMC sampler will transition to mapping character suite histories. Thus the sampler will seamlessly move between using the pruning algorithm and using MCMC to sum over histories.

In summary, we plan to bring together three distinct methodological innovations: the correlated evolution perspective of Pagel *et al.'s* models (Pagel, 1994; Pagel, Meade and Barker, 2004; Pagel and Meade, 2006), the DPP model mixing via MCMC (Neal, 2000), and the data augmentation strategies for general dependent sites models (Robinson et al., 2003; Rodrigue et al., 2005; Rodrigue, Philippe and Lartillot, 2006). This synthesis will dramatically extend the complexity of models available for the phylogenetic analysis of discrete character data.

4 Software implementation

All software funded by the grant will be available under permissive, open source licences. The source code will be hosted on public version control systems (e.g. GitHub) to provide versioning support and cloud-based backups. Binaries will be distributed for Mac and Windows platforms.

Models of morphological character evolution (goals 1 and 2) will initially be implemented in GARLI to enable ML tree searching on fossil data and combined-data matrices. MTH will implement these models in GARLI; an undergraduate will be trained in programming during the research, and the student will perform the initial testing of the implementation.

TAH is the author of DPPDiv (distributed at http://cteg.berkeley.edu/software.html) which implements the MCMC for a DPP approach to divergence time estimation(Heath, Holder and Huelsenbeck,

2012). This software will provide the framework for research into goals 3 and 4 (divergence time estimation and macroevolutionary models). In particular, we will work the optimized "FastDPPDiv" code. That code repository is a version of TAH's code that is being optimized for speed by members of Dr. Alexandros Stamatakis' lab (https://github.com/xflouris/FDPPDIV).

MTH will prototype the implementation of the dependence among characters in year 2. This initial implementation will be built into the code base used in Lakner et al. (2011) to study models of protein sequence evolution. The most effective algorithmic variants will then be added to the DPPDiv package to allow the models of character evolution to be used in conjunction with the other software products of the grant. By the end of the grant, software supporting goals 1 and 2 will be available in GARLI and DPPDiv. DPPDiv will also contain implementation of the analyses for achieving goals 3-5. The most promising analyses that we develop will be ported to the RevBayes software (the successor to MrBayes) project to broaden the user base.

5 Broader Impacts

Broader impacts of this work include training of students and a postdoc, production of open source software, and the creation of a digital teaching labs.

MTH and BSL will co-advise and train a PhD student to become proficient in the areas of trilobite systematics, ML methods, and appropriate programming skills. A second PhD student will be trained (for two summers) in programming and the use of tools for biodiversity informatics related to dissemination of research products.

MTH note: Some information (2 paragraphs) which included discussion of the participation of undergraduates has been removed from this version of the proposal, because we have not received their permission to post the proposal publicly.

The proposed research will result in the development of broadly distributed software for ML and Bayesian analyses of morphological data, and simultaneous estimation of speciation and extinction rates for phylogenies with fossil taxa. Our prototype implementations will be distributed as stand-alone open-source software, but we will also incorporate the models into widely used software to maximize the use of the methods by scientists in the phylogenetics and paleontological communities.

Digital labs – We will create two digital student labs on trilobites. One lab will be designed for students in grades 6-8 in junior high school biology and earth science classes and will introduce them to this major fossil clade. Few junior high schools have extensive collections of fossils. The digital lab will provide a means of bringing this subject into the classroom. The other lab will be designed for college students in undergraduate paleontology classes. Trilobites typically are the subject of a weekly lab in a semester long paleontology course. They are the focus of a major chapter in important textbooks on the morphology of fossil invertebrate phyla (e.g. Clarkson, 1998). The development of the college level lab will facilitate instruction at colleges and universities that lack comprehensive collections of trilobites.

We will feature phylogenetic hypotheses and digital images of the numerous trilobite specimens from the collections of the Division of Invertebrate Paleontology, Biodiversity Institute, University of Kansas (KUMIP). The labs will be made available online through our website, and we will use various professional and educational contacts to make sure they are broadly distributed. The labs will emphasize the ecology, evolution, and morphology of trilobites at different levels of technical detail. In the junior high digital lab, our main pedagogical goals will be to introduce students to the amazing diversity of this now extinct clade. The lab will also discuss the relationship between major environmental perturbations and episodes of diversification and extinction of trilobites. We will use our digital lab to try to spark students' imagination by capitalizing on the fact that trilobites were one of the first bilaterian groups to appear and dramatically diversify in the fossil record. They were incredibly successful, yet now they are entirely extinct. Using this as

an evolutionary microcosm, we will get students to try to understand how groups that seem "too big to fail" can eventually vanish. The labs will explore the significance that this observation has for our understanding of the history of life. We will include discussion about the relationships of trilobites to extant clades and a focus on some of the distinctive character complexes seen in trilobites, especially their remarkable eyes, and the proliferation of spines on the exoskeleton. The college digital lab will delve much more deeply into the subject; it will show insets of the external and internal anatomy of trilobites and consider how we use trilobite morphology to reconstruct evolutionary relationships. Emphasis will also be placed on approaches for identifying individual species and studying ontogenetic sequences. We will also describe what we know about higher-level phylogenetic patterns within the group, and focus on what studies of trilobites have taught us about the tempo and mode of evolution, especially via punctuated equilibria.

BSL already has garnered significant experience with these types of endeavors through his work creating a museum exhibit on trilobites. Translating this experience into this digital lab is very feasible, and will help the PhD students and Dr. Heath gain valuable experience in scientific outreach.

Lab exercises on trilobites have at times focused on rote memorization of morphological features and terms as well as identification of various higher taxa for biostratigraphic purposes. We will not pursue such an approach. Although we will certainly consider the waxing and waning of trilobite clades through time, the emphasis of our labs will be placed squarely and firmly on quantitative analysis of trilobites and evolution.

We note that there is an NSF-funded Research Coordination Network focused on "Advancing Integration of Museums into Undergraduate Programs" (http://www.aim-up.org/). The KU Museum of Natural History is not currently involved in that RCN. However, if this proposal is funded, we will solicit this RCN for feedback on the content digital lab, incorporate suggestions, and make use of the communication channels of the RCN to maximize the impact of our lab material.

Timeline

The schedule for the proposed work is shown in Figure 4. BSL, TAH, MTH, and the PhD student will all be involved in the formulation of the models and analyses. TAH, MTH, and the PhD student will perform the implementation of the inference methods into software. BSL and the PhD student will lead the effort to more fully expand the phylogenetic datasets and to test the behavior of the methods on the trilobite datasets.

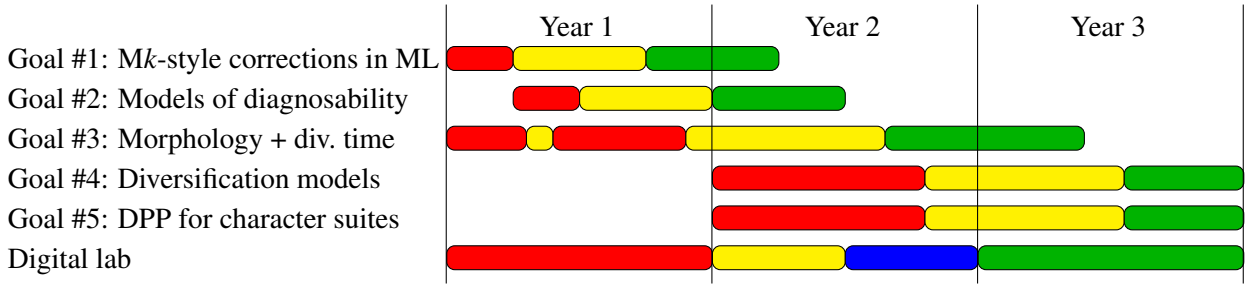

Figure 4: Approximate timeline for the major goals of the project. Red indicates initial research and implementation stage; yellow indicates the testing/validation stage; green indicates the final stage of optimization, publication, and dissemination; blue color indicates soliciting external feedback from educators on the digital lab content.

MTH will implement the character models (goals 1 and 2) into GARLI in the first year of the grant. He has extensive experience working with the GARLI code base. In year two, he will collaborate with the TAH and the PhD student as they spearhead the implementation of the Bayesian software for goals 3 and 4. However his primary contribution in years 2 and 3 will be the development of software for goal 5 (correlated evolution models).

TAH's time commitment to this project is 40% for each of the three years. In the initial year TAH will focus on goal 3 – adding models of rates of morphological character change for divergence time estimation to her DPPDiv software. The PhD student at KU will continue this work by assisting with the implementation of these approaches and by adding the character models into DPPDiv (porting them from the GARLI implementation). In the second year of the grant, TAH will focus on goal 4 (models of diversification and macroevolutionary models). The PhD student will continue to implement these models in year 2 and 3.

Through out the grant, we will thoroughly test and validate the behavior of the methods. The PhD student and undergraduate student will use simulation studies to check for unexpected behavior (either bugs in the implementation or undesirable properties of the models of inference). Many new forms of phylogenetic inference are tested by the authors of the methods (typically computational biologists) on a few simple datasets. The nature of the collaboration in this grant will tightly link a paleontology lab (of BSL) and a computational biology lab (MTH's) via a co-advised student. The large number of well characterized specimens, data on stratigraphy, and varied evolutionary contexts displayed by the Cheiruridae and Olenellina will provide a stringent test of the methods that we develop. This will assure that the software implementations are designed to work on the complexities of real datasets (or at least will prevent us from "overselling" the methods).

In year 3, MTH, TAH, and the PhD student will optimize the most effective variants of the software for goals 3-5 into more widely used Bayesian phylogenetics. TAH is a core developer on the RevBayes project; MTH has code-committing privileges and familiarity with the RevBayes and BEAST2 projects (though he has not contributed substantial code to either project yet).

MTH note: Results from Prior NSF Support deleted.

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