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Diet evolution and clade richness in Hexapoda: A phylogenetic study of higher taxa

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Dryad Digital Repository: Definitions of ecological states, coding of terminal groups and references for raw data [DOI: http://dx.doi.org/10.5061/dryad.6f75v]

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Abstract

Hexapoda, the insects and their relatives, includes over half of all described species. Because large proportions of this diversity cluster within a small set of phytophagous groups, dietary-substrates have been proposed to shape patterns of richness within the clade through antagonistic co-evolution and zones of ecological opportunity. Here we explore these processes in the context of a recent dated phylogeny of Hexapod families. Our results indicate phylogenetic clustering of specialized ecologies such as phytophagy and parasitism, but reveal no consistent associations between the use of particular dietary substrates and clade richness. We also find no evidence that diets expected to promote antagonistic co-evolution are consistently associated with elevated species richness, nor that sister clades differing in dietary state are associated with greater-than-expected differences in richness. We do, however, identify variation in the age of, and transition rates among, dietary states that are likely to play a role in the observed heterogeneity in richness among dietary classes. Based on these findings we suggest remaining circumspect about the generality of adaptive zones based on broad dietary groupings as an explanation for hexapod richness, and suggest that richness heterogeneity may be better explained by origination and transitions rates, and variation within dietary categories.

Introduction

A key issue in macroevolution is how ecology affects speciation and extinction to generate differences in species richness among clades (Schluter 2009). Ecological opportunity is a key potential part of this relationship, and refers to how niche space constrains the richness of clades using these niches (Valentine 1980; Wellborn and Langerhans 2015). Zones of ecological opportunity are challenging to visualize, as they exist in a multi-dimensional volume defined by a combination of many ecological traits (Futuyma and Moreno 1988; Devictor et al. 2010). However, ecological zones may sometimes be approximated by single, simply measured traits, and the distribution of such traits may be studied on phylogenies of radiating taxa (Poisot et al. 2011; Cantalapiedra et al. 2014). For example, the division of niche space into zones of opportunity would be expected to restrict transitions between such zones, resulting in strong phylogenetic conservatism in correlated traits (Cooper et al. 2010; Poisot et al. 2011). Likewise, because zones of opportunity are expected to differ in their control of net diversification rates and carrying capacity, transitions across different ecological zones should correlate with differences in the inferred diversification process and therefore the species richness of transitioning clades (Maddison et al. 2007; Rabosky 2009). Understanding the role of ecology in structuring species richness among clades therefore relies on an understanding of diversification rates, the history of ecological evolution within the group, and an appreciation of the limits to zones of ecological opportunity.

Much of the work on diversification and ecology explores the relationship between species richness and host specialization (Thompson 2009; Poisot et al. 2011). Host specialists, by definition, make use of only part of the resources available in an environment, and therefore zones of opportunity can be occupied by greater numbers of species, potentially resulting in

more species-rich clades (Poisot et al. 2011; Vamosi et al. 2014). Furthermore, antagonistic coevolution, and loss of genetic variation, is expected to result in increased specialization among specialized daughter clades leading to potential long lasting impacts on clade diversification (Ehrlich and Raven 1964). Specialization also imposes macro-evolutionary costs, such as reduced population and range size, which render species more susceptible to extinction, and which may mask or counter the effects of increased diversification rates (Kelley and Farrell 1998; Nosil 2002). Whether and how clades overcome this "paradox of parasitism" (Drake 2003), and how this relates to zones of ecological opportunity, remain major outstanding questions.

A classic system for exploring the relationship between ecology and species richness is the macroevolution of Hexapoda, the six-legged arthropods that include insects and their relatives. Within this clade there is considerable variation among sub-groups in both species richness (Mayhew 2007), and dietary ecology (Grimaldi and Engel 2005), presenting an ideal system for studying relationships between these traits. In addition, due to the typical presence of a feeding nymph or larval stage with limited mobility, there is a long tradition in hexapod studies of using dietary substrates, e.g. phytophagy (Mitter et al. 1988; Winkler and Mitter 2008; Nyman et al. 2010), parasitoidism (Wiegmann et al. 1993), fungivory (Leschen and Buckley 2007), and generalized diets such as detritivory and predation, as proxies for zones of ecological opportunity and therefore controls on clade diversification within the group (Mayhew 2007).

Evidence that use of heterogeneous dietary substrates may promote clade richness in hexapods is based on the widely cited studies of Mitter et al. (1988), Farrell (1998) and Winkler and Mitter (2008). These analyses purported to show that plant feeding (Mitter et al. 1988), and specifically feeding on angiosperms (Farrell 1998; Winkler and Mitter 2008), is correlated with

elevated diversity with respect to sister taxa, across insects as a whole and within Coleoptera (beetles). While this view has become standard in discussions of plant feeding and speciation (e.g. (Nyman 2010) and references therein) there remain a number of outstanding issues associated with this interpretation, including questions surrounding selectivity in the choice of sister group contrasts. Mitter et al. (1988) included only 13 comparisons in their analysis, which due to the state of phylogenetic and taxonomic information then available, show an implicit bias towards larger plant feeding groups. The authors acknowledged this ((Mitter et al. 1988): appendix) and justified the exclusion of small phytophagous radiations on the basis of their playing a marginal role in understanding overall patterns of clade diversification, due to their low diversity and that of their probable sister taxa. Attempts to test this assertion within Coleoptera indicated that such small families may in fact play pivotal roles in the clade's diversification, resulting in an analysis which failed to recover any consistent association between phytophagy and species richness (Hunt et al. 2007). In addition, conflicting evidence from parasitic hexapods challenges the generality of heterogeneous diets for promoting clade diversification (Futuyma and Moreno 1988; Wiegmann et al. 1993).

In recent years there has been a steady increase in the phylogenetic information available for Hexapoda e.g. (Trautwein et al. 2012; Misof et al. 2014), and in techniques for assembling such data into increasingly comprehensive frameworks for the group e.g. (Rainford et al. 2014). As a result it is now possible to extend the methodologies used by Mitter et al. (1988) and others to consider a more inclusive view of hexapod diversification. The aims of this study are thus: a) to summarize the phylogenetic distribution of diets across higher insect taxa based on a consistent dietary classification (see Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v (Rainford and Mayhew 2015)) and evaluate what this

implies about the historical patterns of dietary acquisition and loss, b) to demonstrate if there is phylogenetic conservatism in diet across the broad array of hexapod taxa as a prerequisite to a long term macro-evolutionary association between diet and species richness, and c) to investigate the association between net diversification and dietary ecology, specifically whether the use of particular substrates is correlated with elevated or depressed richness among hexapod clades, and if consistent patterns occur among the set of diets that are expected to promote antagonistic co-evolution.

Methods

Underlying this study is a dated topology of Hexapoda, including 874 terminal taxa covering 903 of the approximately 1100 extant hexapod families (Rainford et al. 2014). Whilst clearly a phylogeny so inclusive will never be error-free, and some regions whilst plausible, are only weakly supported, this topology includes all clades highly supported by previous work at the level of hexapod families (Rainford et al. 2014), and is broadly consistent with recent opinions regarding the deep structuring of higher taxonomic relationships (Trautwein et al. 2012; Misof et al. 2014). We therefore propose it as the best current working basis for a broad and inclusive comparative study of hexapod diversification. Accompanying this tree are estimates of described species richness for terminal groups taken from recent encyclopedia and related sources (references in (Rainford et al. 2014): Supplementary Material).

Dietary ecology for terminal groups was taken from published descriptions (Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v) and categorized according to predominant substrate use among subfamilies or comparable groups. The substrates used include fungivory, detritivory, phytophagy (herbivory), predation, parasitoidism, and ecto-parasitism as

well as non-feeding and liquid-feeding adults (Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v). Diets were coded separately for juveniles and adults, with most non-metamorphosing taxa assumed to maintain the same ecology throughout the lifecycle. Omnivorous taxa or taxa in which subfamilies varied in predominant ecology were coded as mixed states (Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v). In order to reflect differences in previous classifications regarding the treatment of marginal diets, such as whether to classify xylophagy and/or pollenivory under phytophagy or detritivory (Mitter et al. 1988; Hunt et al. 2007), and whether to group carnivorous parasites (parasitoids, ecto-parasites and other blood feeding taxa) as a single category (Wiegmann et al. 1993), we developed three distinct coding schemes, details of which are provided in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v. Our favored scheme, emphasizing larval/immature diets, is denoted "Larval Raw". A scheme that more closely corresponds to the categories used in previous sister-group studies is henceforth "Larval Modified" (in parentheses; see Dryad Digital Repository http://dx.doi.org/10.5061/dryad.6f75v). Finally, a scheme based on the ecology of adult taxa is henceforth- "Adult".

To assess the degree to which ecological states demonstrated non-random phylogenetic structure across terminal groups, e.g. due to clustering or over-dispersion, we used Phylocom (Webb et al. 2008) to calculate two indices; net relatedness index (NRI- measuring total phylogenetic distance between taxa with particular ecologies) and nearest taxon index (NTI- measuring mean distance to nearest neighbor sharing a particular diet) relative to 999 randomized tip permutations. Given that hypothesized zones of ecological opportunity implicitly assume long-term associations of clades with particular dietary substrates (Irwin et al. 2012), the presence of phylogenetic conservatism can be regarded as evidence consistent with such models.

Taxa with mixed coding states were treated as contributing to all relevant indices and taxa with for which no ecological information could be obtained (denoted by "?" in Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v) as contributing to all studied indices, so as to minimize any biasing effect this lack of data might have on the analyses.

As the basis for subsequent sister-group comparisons (see below) we reconstructed ancestral dietary states under parsimony (using Mesquite (Maddison and Maddison 2011)), and maximum likelihood (ML) using the hidden rates Markov model rayDISC (R package; corHMM (Beaulieu et al. 2013)). For the ML reconstruction of the "Adult" dataset the rarity of some ecologies, e.g. fungivory, resulted in an overexpression at deep nodes within the phylogeny, (see (Nosil 2002)). To resolve this we constrained the root state for this reconstruction to detritivory in order to match the parsimony reconstruction. We converted reconstructed probabilities into discrete states using a threshold approach; with all nodes where a single state represented greater than 0.7 of the total probability referred to this state, and the remaining nodes referred to mixed-states encompassing all traits present with a probability of at least 0.05. These values were selected to maximize similarity with previous studies in inclusion of clades showing strong dominance of particular diets, while maintaining ambiguity where ancestral states are uncertain.

Comparisons of richness across sister clades with divergent ecologies were estimated for each novel origination of a trait on our tree following Mitter et al. (1988). As with these authors our compared richness values subtracted any members of the focal clade belong to terminal taxa lacking the ecology of interest or members of the sister group processing the focal ecology, including within mixed states (henceforth corrected richness). For the purposes of corrected richness, we did not apply subtractions for dietary variation within our terminals due to limits in describing diet and species richness in clades below the family level for many ecologically

diverse groups (see discussion). Contrasts where the ecology of either taxon was unknown were excluded. An example of a set of contrasts with corrected richnesses for phytophagous groups (ML reconstruction) is shown in Table A3 (Online Appendix).

Corrected richness values were compared using the sign binomial test (Farrell et al. 1991) and "species diversity contrast" (SDC) methods that incorporate the magnitude of the diversity contrast between groups (Vamosi and Vamosi 2005). Three SDC statistics were calculated (using Python script "Systers"; (Hardy and Cook 2010)), represented distinct approaches to scaling richness comparisons: raw contrast (Wiegmann et al. 1993), proportional contrast (Barraclough et al. 1995) and log contrast (Barraclough et al. 1996). Following Vamosi and Vamosi (2005), sample size dependent statistical tests were applied to these statistics; with very small samples (<6 comparisons) analyzed using a randomization test of matched pairs, and larger sets compared using a Wilcoxon non-parametric test or its normal approximation (for > 20 comparisons). In this study we did not use the well-known Slowinski and Guyer (1989) test for diet contrasts following evidence of an elevated type one error rate when multiple comparisons are combined within a single test (de Queiroz 1998; Vamosi and Vamosi 2005). Likelihood models of trait-dependent diversification processes, e.g. BiSSE (Maddison et al. 2007), were not used here, as current implementations rely on simulations of species complete topologies for parameter estimation on trees of higher taxa, resulting in exponential growth in time and memory requirements, that render such methods computationally intractable on the scale of Hexapoda (FitzJohn et al. 2009). We further question whether such approaches, which estimate uniform speciation and extinction rates across whole dietary classes, are appropriate for clades where there is clearly enormous rate heterogeneity across subgroups (e.g. Rainford et al. 2014).

To assess the hypothesis that use of biochemically heterogeneous states, i.e. those expected to promote antagonistic co-evolution, might collectively act as drivers of species richness in hexapods we combined these states (phytophagy, parasitoidism, ectoparasitism and fungivory) into a single character, which we term "potential for specialization" or PS. Ancestral reconstruction of the PS character was conducted as above and corrected richness contrasts calculated on novel originations vs. "generalized" sister taxa using the SDC methods described above.

We also explored the idea that related clades occupying different ecological zones (i.e. processing different diets) should be associated with larger than expected absolute differences in richness, arising from differences in the control of net diversification and carrying capacity. To do this we calculated standardized differences in richness at each node in the tree (standardized relative rate difference; stRRD, defined as the absolute contrast in log richness of the two descendant clades) using the trickle down protocol of Davies et al. (2004) as compensation for phylogenetic nesting. The latter uses single-contrast Slowinski-Guyer (1989) tests to identify nodes associated with significant differences in the species richness of descendant clades and then compensates for such shifts for comparisons of more deeply nested nodes (Davies et al. 2004). Our implementation differs from previous work in that where the richnesses of both descendant clades differ significantly from that of the outgroup, we use the sum of the two descendant richnesses for more deeply nested comparisons, thus avoiding corrections where the direction of richness change is ambiguous (Davies et al. 2004). Following standardisation we calculated the mean stRRD value for nodes where ecology was divergent between descendent clades, including mixed states and compared this with the distribution of means of 1,000 sets of equal length, drawn at random from nodes of the tree.

Results

Based on our favored, "Raw Larval" classification, just over half of all hexapod species belong to families that contain at least some plant feeding taxa (527,000 species of the 1,038,000 estimated described taxa within the clades present on the discussed tree; (Rainford et al. 2014)). This compares with approximately thirty percent represented each by families including detritivorous and predatory representatives (330,000 and 322,000 species respectively), nineteen percent for fungivory (194,000 species), thirteen percent for parasitoids (136,000 species) and less than one percent ecto-parasites (7700 species). By comparison adult hexapods are dominated by liquid feeding taxa, which comprise the majority of adult Holometabola (411,000 species), with approximately equal proportions of detritivorous (301,000 species), phytophagous (351,000 species) and predatory groups (260,000 species), and minority representation of fungivores (167,000 species), non-feeding groups (116,000 species) and blood-feeders (39,000 species). Note that the percentages given here incorporate terminal taxa with mixed ecologies into each of the relevant dietary categories hence they exceed one hundred percent.

Significant phylogenetic clustering of fungivory, phytophagy, parasitoidism and ectoparasitism was observed under both the NRI and NTI metrics for the "Raw Larval" and "Larval Modified" coding systems (Figure 1, Online Appendix: Table A1). This implies that on average, taxa with these ecologies tend to be closely related to other taxa with the same diet (Figure 2). This pattern was not observed in detritivory and predation, both of which show non-significant trends towards over-dispersion in both larval datasets with respect to NRI. For adult ecologies, significant clustering was observed for fungivory, blood-feeding and non-feeding diets while predators, detritivores and plant feeders showed no significant trend with respect to NRI.

Both parsimony and ML reconstructions identified detritivory as the ancestral and most widespread larval ecology within Hexapoda (Figure 2), although under ML some degree of fungivory is inferred in the early insect radiation, based on the diet of the poorly known basal order Protura (Pass and Szucsich 2011). The two methods identified broadly similar patterns in resource use across the tree, with most ordinal groups showing strong conservatism with respect to diet, resulting in a pattern dominated by a small number of well characterized radiations, for example that of plant feeding within Lepidoptera and parasitisoidism within Hymenoptera (Grimaldi and Engel 2005).

Disagreement between reconstruction methods typically reflects taxa showing high degrees of ecological lability, e.g. Coleoptera, where ML identifies fungivory as the ancestral ecology, and multiple originations of detritivory, whereas under parsimony this pattern is reversed. This reflects genuine ambiguity regarding the deep topology of the order, as well as the close relationships of many families associated with both dietary states (e.g. with wood-boring or soil-living lifestyles) (Hunt et al. 2007). Likewise basal members of the fly suborder Brachycera show conflict regarding the origination of predation, which under ML was recovered as multiple independent origins from a detritivorous ancestor in Asiloidea, Empidoidea and Tabanomorpha, as opposed to a single origin, with a return to detritivory in Stratiomyomorpha and Cyclorrhapha observed under parsimony (Marshall 2012).

The implemented ML model for the Larval Raw dataset allowed all transition rates to vary independently (all rates variable AICc = 1182.3, vs. equal rates AICc= 1244.2, a significant difference of 61.87 likelihood units). The highest-obtained transition rates were recovered between fungivory and detritivory (Table 1), reflecting the widespread nature of these diets and frequent transitions within Coleoptera and Diptera (see above). Transitions between detritivory

and predation also occur at high rates, being particularly common among freshwater taxa, e.g. caddisflies and mayflies where multiple parallel origins of predatory larvae exist in various families. Ecto-parasitism is identified as a dead end with respect to ecological diversification with no examples of the emergence of other ecologies within a primitively ecto-parasitic group (transition probabilities equal zero). Note that ecto-parasitism in larval hexapods is extremely rare and restricted to four clades, including one ancient origination in Pthiraptera (lice; whose age is highly uncertain (Rainford et al. 2014) due to lack of suitable fossils (Grimaldi and Engel 2005) and accelerated rates of genomic evolution (Trautwein et al. 2012)), and three further events occurring among young terminal groups with modest extant diversity. Other transitional dead-ends also appear: there are no direct transitions between fungivory and ecto-parasitism or from parasitoidism to fungivory or detritivory. In the later case this result is dependent on the coding of pollenivory based on the results of the Larval Modified dataset (Online Appendix: Figure A1, Table A2).

The accumulation curve of dietary originations for the ML reconstruction of the Larval Raw dataset demonstrated differences in the relative timing and rates of origination between diets that collectively may contribute to their respective differences in richness (Figure 3). These series reveal certain ecologies including fungivory, phytophagy and predation as appearing early in the history of the group and undergoing approximately consistent rates of origination throughout the history of Hexapoda. This contrasts with patterns in detritivory, and parasitoidism both of which are strongly skewed, such that the majority of originations occur within specific time intervals (respectively the Middle and Late Mesozoic). Friedman tests using the number of originations within 50Ma bins reveal significant differences in the origination rate across dietary categories (maxT = 3.068, p-value = 0.02622). Post-hoc analysis; (Galili 2010), identified

significance as driven by a large contrast between the predatory and ecto-parasitic dietary classes (p=0.0263) as well as a marginally non-significant contrast between ecto-parasitism and phytophagy (p=0.0574). By comparison the ML reconstructed Larval Modified dataset (where ecto-parasitism and parasitoidism are combined) revealed no comparable differences between binned ecological categories (maxT = 1.6813, p-value = 0.446) suggesting that these differences arise from splitting these two distinct forms of carnivorous parasitism into discrete categories. Likewise there were no significant differences in the binned counts among the ecologies in the Adult dataset (ML reconstruction; maxT = 2.4076, p-value = 0.195).

Compared with the larval phase, information on the extent and significance of adult feeding within many hexapod groups is relatively uncertain, resulting in poorer documentation and fewer records of adult diet. As a result, reconstructions from our adult dataset are subject to extrapolation errors under ML associated with rare ecologies (see above) and high degrees of conflict were observed between reconstruction techniques (Figure 4). Major regions of conflict include: the ancestral state of Mecopterida (including Trichoptera, Lepidoptera, Diptera, "Mecoptera" and Siphonaptera) (Trautwein et al. 2012), the relative importance of fungivory in the diets of adult beetles (many of which are polyphagous relative to their larval stage), and ancestral diets within Heteroptera.

Sister group comparisons failed to show any significant effect of any dietary ecology on species richness. The only potential exception was the reconstruction of detritivory under parsimony, which showed a significant trend with respect to Raw contrasts towards increased richness (Table 2), primarily driven by the novel origination of detritivory in Cyclorrhapha (Diptera) (conflicting with ML reconstruction). The analysis of the Larval Modified dataset produced similar results indicating that this lack of previously identified relationship was not

simply a manifestation of differences in the coding system between this work and previous studies. Similarly no significant trends in richness association were observed with respect to adult ecology.

The reconstructed history of the PS character state was identical under parsimony and ML methods and corresponded to the major specialized groups previously described (Figure 2, Online Appendix: Figure A2). The associated transition matrix for the ML model identified a marginally significant bias in transition rates towards the evolution of more specialized ecologies (0->1: 0.00072 myr^{-1} vs. 1->0 0.00035 myr^{-1}, AIC= 526.51, vs. an AIC of 528.19 for an equal rates model). Sister group comparisons between PS and non-PS groups failed to recover any evidence for the trait promoting diversity with exactly half of the test comparisons running contrary to the view (24 of 47 instances, Sign Test *p* value= 0.5106, Wilcoxon Tests: Raw Contrasts; W=504, *p*= 0.52893, Log Ratio Contrasts; W=545, *p*=0.84479, Proportional Contrasts; W=524, *p*=0.67595).

When we compared nodes where ecology diverges for the "Larval Raw" and "Larval Modified" datasets there was no significant trend in terms of greater than expected contrasts at nodes with divergent ecologies. For the "Larval Raw" data, the mean estimate of the stRRD value associated with nodes showing ecological divergence was 2.1245, corresponding to a *p* value of 0.2741, assuming a normal distribution of the log means of the 1000 randomly sampled node sets (mean of random samples 2.061; sd 0.1058; Shapiro-Wilk normality test; $W = 0.9994$, p-value = 0.0948). For the "Larval Modified" mean stRRD = 2.116, *p* value= 0.2898, (mean of random samples 2.057; sd 0.1072; SW test; $W = 0.999$, p-value = 0.562).

By contrast in the "Adult" dataset there was a marginally significant trend towards larger contrasts, estimate of stRRD = 2.252, *p*= 0.0484**,** (Mean of random samples 2.0572; sd 0.1175;

SW test; $W = 0.998$, p-value = 0.296). It is unclear whether this difference between datasets simply reflects the presence of more character states in the Adult dataset (thus generating more divergent nodes) or if it is the influence of a small number of deep dietary shifts, e.g. the transitions to predominantly non-feeding or liquid feeding diets within Holometabola. Based on these results we conclude that one of the basic assertions of a ecological zone model, that groups with divergent ecologies should show greater than average differences in richness (after standardization to render contrasts independent), cannot be demonstrated as holding at the resolution of hexapod families

Discussion

This study explores the association between dietary substrate and hexapod richness via adaptive zones of opportunity. Surprisingly, no association was found between particular dietary ecologies and net diversification, in contrast to previous findings relating to phytophagous insects. Also, our specialized and generalized diets do not consistently promote differences in species richness. Nor are nodes involving dietary shifts attributed greater shifts in diversification than random nodes. There is, however, evidence for strong phylogenetic conservatism of specialized diets but not generalized diets. Finally, there are differences in transition rates and origin times between diets, with a tendency towards evolution of diets associated with co-evolution and host specialization. Below we interpret these findings and their consequences for understanding how diet affects hexapod diversification.

Our original motivation was to explore how hexapod diet acts as a proxy for zones of ecological opportunity (Mayhew 2007). The association of diet with zones of ecological opportunity is expected to result in phylogenetic clustering, arising from restricted transitions

between diets (Cooper et al. 2010; Poisot et al. 2011). We observed phylogenetic conservatism in the use of biochemically and mechanically heterogeneous resources such as fungivory, phytophagy and parasitoidism. Such clustering is weaker for detritivory and predation (Figure 1, Table A1). Strong phylogenetic conservatism in hexapod diets, e.g. herbivorous insects, is widely acknowledged, e.g. (Ehrlich and Raven 1964; Mitter and Farrell 1991; Futuyma and Agrawal 2009), although comparisons across multiple substrates are rare.

Phylogenetic conservatism in the use of heterogeneous substrates may be generated by a requirement to overcome with host defenses (Mitter and Farrell 1991; Futuyma and Agrawal 2009) and skewed nutrient content (Mattson 1980; Douglas 2009), which may restrict colonization of these resources by novel hexapod lineages. By contrast, intermediate stages such as scavenging, incidental predation of cohabitants, and cannibalism, may serve to lower such barriers for originations of "generalist" diets (Coll and Guershon 2002), allowing their adoption by a wider range of clades (Figure 3), and as a result, reduced phylogenetic clustering across Hexapoda. The higher transition rates from generalized to specialized diets also supports this interpretation, mirroring previous species-level studies within dietary groups (Nosil 2002; Nosil and Mooers 2005). These findings suggest that "specialized" ecologies are consistent with being zones of opportunity, at the family level, in contrast to "generalized" diets. However, this pattern alone is insufficient to define dietary adaptive zones, which are also contingent on the association of different diversification processes with particular diets (Maddison et al. 2007; Rabosky 2009).

Contrary to previous publications, our work finds no evidence that plant-feeding groups are consistently more species rich than their sisters; a view which has been very influential (e.g. (Grimaldi and Engel 2005; Nyman 2010) and references therein). We think it unlikely that our

lack of evidence reflects a lack of power, as our sampling of phytophagous clades was more comprehensive than in previous studies (13 sister comparisons in Mitter et al. (1988); vs. 25; Larval Raw or 26; Larval Modified here). Instead these differences probably arise from earlier selective sampling towards representation of larger and phylogenetically better known plant-feeding groups ((Mitter et al. 1988): appendix), and heterogeneity in the macro-evolutionary dynamics of phytophagous lineages. Our total number of parasitism comparisons is identical to that of previous studies (15; (Wiegmann et al. 1993)), although the identity of the groups shows minor differences (Rainford et al. 2014). In agreement with previous work, our analysis fails to show a consistent association of parasitism and species richness, including where invertebrate and vertebrate parasites are treated separately (as in Larval Raw).

Our study does not distinguish between phytophagous clades feeding on angiosperms and gymnosperms (Farrell 1998; Winkler and Mitter 2008), because hexapod families often include species feeding on both plant clades. The idea that host clades may produce different diversification dynamics, has been discussed extensively (Winkler and Mitter 2008; Nyman 2010), e.g. the Cretaceous radiation of angiosperms expanding the ecological space available for plant-feeding clades (Labandeira and Eble 2000; Grimaldi and Engel 2005). However, testing this assertion is challenging due to uncertainties in the timing of hexapod diversification (Rainford et al. 2014; Misof et al. 2014), the extended emergence of angiosperms prior to their appearance in the fossil record (Clarke et al. 2011), and because insect radiations may be decoupled from the radiations of their hosts e.g. (McKenna et al. 2009). As such it is beyond the reach of the presented datasets.

Our analysis also fails to identify consistent differences in net diversification between specialist and generalist diets (represented here by our PS character). Likewise, sister taxa that

differ in ecology do not experience significantly increased differences in richness, as would be expected if diets represent different adaptive zones (Rabosky 2009). This opens up alternative perspectives on the controls on hexapod diversification (see below).

Differences in the richness of dietary groups can alternatively be explained by historical factors in the evolution of hexapod diet. For example, the timing of originations, such as bias towards post-Mesozoic originations in parasitoidism (Labandeira and Eble 2000; Grimaldi and Engel 2005), may limit the richness of such clades when compared with older (e.g. phytophagous) lineages (Figure 3). Time-lagged evolution of suitable hosts may also limit the richness of ecto-parasites (Whiting et al. 2008), while other diets such as fungivory, detritivory and phytophagy have undergone many parallel and ancient origins which may partly account for differences in their richness (Figure 3) (Grimaldi and Engel 2005).

The flipside of origination is extinction; however evidence regarding the latter is limited in phylogenetic studies of extant taxa (Labandeira and Eble 2000). Insect fossils, whilst providing direct evidence of the extinction of higher taxa, provide little evidence regarding the diets of extinct groups (Grimaldi and Engel 2005). One major, (probably) phytophagous group, the Palaeodictyoptera, went extinct at the Permo-Triassic mass extinction (Labandeira 2006; Labandeira 2013), however the implications of this for modern plant-feeding clades remains unclear, and to date no fossil studies have attempted to compare the extinction rates of different dietary categories.

The frequency of transitions between different dietary groups is another historical factor potentially affecting the richness of different diets. Ecto-parasitism originates at a very low rate compared to other dietary categories, as does parasitoidism (Table 1). Some transition rates to the other dietary categories are much higher; for example from fungivory to detritivory,

fungivory to phytophagy, fungivory to predation, and detritivory to predation (Table 1). The high rates associated with transition away from "generalized" ecologies mirrors recent findings with respect to mammalian dietary evolution (Price et al. 2012), although contrary to the latter it seems unlikely that "generalized" categories in hexapods represent unstable intermediates between specialized diets (see discussion of omnivory in (Coll and Guershon 2002; Price et al. 2012)). Ecto-parasitism appears to be an evolutionary dead-end in hexapods (Kelley and Farrell 1998), resulting in no further transitions to other dietary substrates and likewise, at the studied resolution, there are no transitions from fungivory or phytophagy to ectoparasitism (Table 1). This may be due to extreme differences in nutrient content (Mattson 1980), the requirements of appropriate nutritional symbionts (Douglas 2009) and limited opportunity for the establishment of long-term insect-host associations (Lehane 1991; Balashov 2006). Thus the data presented here suggest that, even in the absence of consistent differences in net diversification between diets, the historical pattern of originations and transitions between diets goes some way towards explaining the heterogeneity in richness between different dietary categories. On their own however they fail to provide an explanation for the exceptional richness of Hexapoda that the adaptive zones hypothesis potentially provides.

Given these findings, what is the role of dietary adaptive zones in hexapod diversification? One possibility is that the real impact of diet is masked by uncertainties in hexapod taxonomy, phylogeny and ecological description. Discussion of the problems of monophyly and richness estimates can be found in Rainford et al. (2014). Phylogenetic uncertainties in hexapod relationships (Trautwein et al. 2012; Misof et al. 2014) could in principle bias the results, particularly if small clades with divergent diets, whose phylogenetic placement is generally less certain, have been systematically wrongly placed next to taxa with

greater richness than that of their true sister groups. However, we currently have no reason to suspect such a bias, and thus expect to maintain signal across the implemented tests. Overall, the presence of numerous, and previously neglected, species poor phytophagous taxa give reason to remain circumspect on the generality of the findings of Mitter et al. (1988) and positive and sensible results found elsewhere in this paper suggest substantial signal regarding diet and species richness is present within our dataset.

Unseen ecological variation within families may also bias our results. However, available descriptions severely limit analysis at finer taxonomic scales: there are often no species richness estimates below the family level, or published descriptions may not attribute observed variation in diet to particular sub-taxa, particularly where observations are known for only a few species. Within-family phylogenetic uncertainties are also limiting; for example previous work involved contrasts using subfamilies of Scarabeidae and Coccinellidae (Coleoptera) (Mitter et al. 1988; Hunt et al. 2007), the sister groups of which have been disputed by subsequent phylogenetic work, e.g. (Smith et al. 2006; Magro et al. 2010). It is possible that compiling all sets of ecological contrasts would collectively reveal different patterns to those described here, however this would still leave considerable heterogeneity in diversification within dietary groups to be explained (Mayhew 2007).

As this study draws extensively on the results of ancestral reconstruction it is important to acknowledge the sensitivity of these techniques to model misspecification, rapidly evolving traits, widespread convergence and transitions via intermediate states which may be lost in extant representatives (Cunningham et al. 1998; Nosil 2002; Beaulieu et al. 2013). The uncertainties surrounding historical hexapod diets and the timings of dietary transitions (Labandeira 2006; Labandeira 2013) restrict the extent to which we can test the impact of these limitations.

However there tends to be close agreement between the transitions shown here and previous broad historical hypotheses (see Grimaldi & Engel 2005).

Sister clade comparisons of species richness explore only the sum of speciation and extinction impacting on focal taxa. Approaches that attempt to tease apart these processes e.g. BiSSE (Maddison et al. 2007) have become increasingly popular and may play a role in resolving some of the ideas discussed here. Note however that in current implementations these procedures have their own limitations (see methods). Very recently the idea has been proposed of using global inference of diversification processes in combination with tree pruning to describe the subset of diversification rates associated with procession of a particular trait e.g. (Weber and Agrawal 2014). This is an idea that holds considerable promise for future work, however once again there are issues relating to its implementation for Hexapoda.

Leaving aside the above methodological issues, there remains the possibility that the observed lack of association between diet and diversification reflects real features of hexapod evolution. This implies that, rather then each diet being linked to a particular diversification process, different clades using the same substrate respond in different ways. In other words, substrate-based classifications, such as that applied here, may be poor approximations for the real zones of ecological opportunity that have shaped hexapod diversification. Instead we should consider how other features of diet or hexapod traits may have shaped the macroevolution of diversifying clades (Mayhew 2007). A simple example would be host clade specific diversification, such as between gymnosperms and angiosperms (see discussion above). However, other features, such as differences in spatial context, e.g. between terrestrial and aquatic taxa (Hunt et al. 2007), and the role of ecological co-variates such as body size and

dispersal capacity, may modify the effect of diet on clade diversification (Isaac et al. 2005; Phillimore et al. 2006).

A further possibility is that differences among taxa in their ability to transition between ecological zones may have consequences for their relative diversification (Dodd et al. 1999). Evidence for evolvability as a correlate of richness remains limited (Dodd et al. 1999), and is subject to theoretical issues regarding the underlying model of character change (Ricklefs and Renner 2000; Silvertown et al. 2000). These, as well as data restrictions due to our incomplete sampling of hexapod diets (see above), mean that we do not incorporate such ideas into this study, although we acknowledge the potential for future analysis in exploring these ideas.

One potential source of heterogeneity within diets is the ecological feeding guild (Simberloff and Dayan 1991), i.e. the manner in which taxa utilize a particular resource. Evidence for the importance of guild-specific processes can be found in community assembly studies (Novotny et al. 2010; Novotny et al. 2012), as well as differences in the fossil dynamics of higher taxa (Labandeira 2006; Labandeira 2013). However, to date few guilds have been explicitly explored in terms of diversification e.g. leaf-mining; (Connor and Taverner 1997), galling (Hardy and Cook 2010). Some others, such as the distinction between idiobiont (which restrict host development from the point of parasitism) and koinobiont (which must deal with active defense by the developing host) parasitoids, have been subject to intensive speculation e.g. (Hawkins 2005; Santos and Quicke 2011) and warrant serious consideration in future studies.

Since the work of authors such as Gould and Calloway (1980) and Mitter et al. (1988) the broad emphasis of trait-based diversification studies has predominantly been on testing a-priori hypotheses regarding the association of traits with patterns of diversification. While acknowledging the power of this approach, there is a need to be rigorous in discussing the

relationships between studied proxies (e.g. dietary substrate) and the processes of interest that may have acted to shape clade diversification (e.g. host specialization and zones of opportunity) (de Queiroz 2002; Vamosi et al. 2014). In our analyses we group a set of ecologies potentially associated with promoting co-evolution and host specialization, under the expectation that these might show common patterns of clade diversification (our PS traits). However, a clear definition of "specialization" that is applicable when comparing different ecologies remains lacking (Devictor et al. 2010; Poisot et al. 2011), rendering comparisons between diets ambiguous (Giller 1996; Nyman 2010). Attempts to resolve this issue through metrics of specialisation (e.g. based on the number or phylogenetic diversity of host lineages used by taxa (Forister et al. 2015), or on measures of interspecific competition within communities (Kaplan and Denno 2007)), have yet to be widely adopted and remain restricted to single dietary classes (Poisot et al. 2012). There is therefore a need to use language rigorously to describe these interactions and their relationship to theoretical models of niche divergence ((Vamosi et al. 2014) and references therein).

To conclude, the work presented here suggests that, while some diets show strong conservatism at the level of hexapod families, and the origination dates and transition rates between different broad diets go some way towards explaining their heterogeneity in species richness, evidence for differential diversification processes operating within these substrate-based categories is lacking. It seems likely that by the restriction of discussion to arbitrary and subjective classifications we are failing to appropriately account for the different processes that may be responsible for shaping clade richness and how these relate to our measured proxy traits (Nyman 2010). Understanding this linkage will require a combination of detailed ecological study, as well as further investigation into the macro-evolutionary process with a view towards defining appropriate hypotheses to test with comparative methods. Ultimately thereby we may

establish a more “insect’s eye” view of adaptive landscapes and thus enhance our understanding of the processes that drive diversification within the clade.

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Online Appendix: Supplemental Information

Table A1: Clustering analysis of different character states inferred for the different coding systems.

Coding	Ecology	MPD	MPD.r	MPD.sd	NRI	p	MNTD	MNTD.r	MNTD.sd	NTI	p
Larval Raw	Fungivory	696.96	779.35	11.24	7.33	<0.001	341.46	373.91	15.66	2.073	0.0197
Larval Raw	Detritivory	781.83	781.50	5.45	-0.0597	N.S.	265.96	294.98	6.704	4.329	<0.001
Larval Raw	Phytophagy	730.25	792.10	5.60	11.04	<0.001	250.07	308.86	7.570	7.766	<0.001
Larval Raw	Predators	796.34	792.55	6.50	-0.584	N.S.	277.063	319.32	8.730	4.840	<0.001
Larval Raw	Parasitoids	657.03	789.18	11.20	11.80	<0.001	294.76	378.79	15.90	5.285	<0.001
Larval Raw	Ectoparasites	743.37	783.94	16.82	2.412	0.0124	386.16	438.73	25.53	2.059	0.0206
Larval Mod	Fungivory	698.48	779.32	11.28	7.166	<0.001	344.67	374.66	15.75	1.905	N.S.
Larval Mod	Detritivory	787.41	781.36	5.554	-1.089	N.S.	269.30	296.26	6.844	3.941	<0.001
Larval Mod	Phytophagy	726.20	791.83	5.831	11.256	<0.001	250.05	311.67	7.853	7.846	<0.001
Larval Mod	Predators	796.94	792.79	6.478	-0.6403	N.S.	278.00	320.46	8.816	4.817	<0.001
Larval Mod.	Parasites	704.64	790.12	9.324	9.1671	<0.001	283.24	355.68	12.81	5.657	<0.001
Adult	Fungivory	677.92	779.89	12.64	8.0641	<0.001	347.71	391.23	18.22	2.388	0.0091
Adult	Detritivory	791.08	779.12	7.215	-1.6581	N.S.	313.27	318.95	9.129	0.622	N.S.
Adult	Phytophagy	788.84	782.84	9.091	-0.66	N.S.	320.58	346.67	12.10	2.157	0.0156
Adult	Predators	797.92	786.07	7.640	-1.5506	N.S.	290.80	331.24	10.18	3.974	<0.001
Adult	Blood feeders	731.18	788.42	12.41	4.6106	<0.001	326.60	399.86	18.70	3.918	<0.001
Adult	Non feeding	734.51	792.06	9.852	5.8416	<0.001	276.80	371.21	14.45	6.534	<0.001
Adult	Nectivory	653.43	793.85	5.064	27.731	<0.001	219.49	298.83	6.714	11.82	<0.001

Note- Column headings: MPD (MPD.r, MPD.sd)- Mean phylogenetic distance of taxa possessing a particular ecology in the data set, and the mean and standard deviation of the implemented randomizations respectively, NRI- net relatedness index, MNTD (MNTD.r, MNTD.sd)- Mean Nearest Taxonomic distance of dataset and mean and standard deviation of value of the implemented randomizations respectively, NTI- Nearest Taxon index. MPD and MNTD are given in millions of years (the unit of branch length of the underlying phylogeny). NRI and NTI are dimensionless ratios, defined on the difference of the observed and mean expected values divided by the standard deviation of expected values, positive values referring to clustered data (Webb et al. 2008). P-values are calculated based on a two-tailed test.

Table A2: Overall Likelihood and transition rates per million years inferred for the optimal ML model of Larval Modified dataset.

	Fungivory	Detritivory	Phytophagy	Predators	Parasites (Combined)
Fungivory	NA	0.002054	0.00068	0.00063	0.00019
Detritivory	0.00015	NA	0.00037	0.00074	0.00024
Phytophagy	0.00033	0.00015	NA	0.00003	0.00010
Predators	0.00007	0.00016	0.00021	NA	0.00014
Parasites(Combined)	0.00	0.00014	0.00009	0.00011	NA

Note- Overall LnL: 561.30, AIC: 1162.61, n. taxa: 874. Models are denoted as transition rates from rows to columns.

Table A3: Sister group comparisons for the Larval Raw under a ML reconstruction.

Node Age /Ma	Clade A	Corrected Richness A /n. species	Clade B	Corrected Richness B /n. species
259.6	Phasmatodea	2940	Embioptera	337
210.7	Acridomorpha (Orthopera)	8318	Tetrigidae, Thericleidae (Orthoptera)	1246
117.5	Thericleidae (Orthoptera)	220	Tetrigidae (Orthoptera)	1246
389.7	Hymenoptera	34021	Remaining Holometabola	342555
53.3	Mordellidae (Coleoptera)	1500	Meloidae (Coleoptera)	3000
216.6	Curculionoidea (Coleoptera)	61851	Chrysomeloidea, Cryptophagidae, Kateretidae, Laemophloeidae, Propalticidae, Phalacridae, Monotomidae, Erotylidae, Languriidae, Passandridae, Helotidae (Coleoptera)	9224
154.3	Chrysomeloidea (Coleoptera)	62619	Cryptophagidae, Kateretidae, Laemophloeidae, Propalticidae, Phalacridae, Monotomidae, Erotylidae, Languriidae, Passandridae, Helotidae (Coleoptera)	4666
72.4	Kateretidae (Coleoptera)	95	Cryptophagidae (Coleoptera)	600
80.51	Byturidae (Coleoptera)	24	Biphyllidae (Coleoptera)	200
92.8	Artematopodidae (Coleoptera)	45	Hydraenidae (Coleoptera)	1600
163.4	Pleocomidae (Coloptera)	50	Lucanidae (Coleoptera)	1489

240.5	Byrrhidae (Coleoptera)	430	Buprestoidea, Dascilloidea, remining Byrrhoidea (Coleoptera)	3871
102.8	Buprestidae (Coleoptera)	14700	Heteroceridae (Coleoptera)	300
59.5	Tephritidae (Diptera)	4716	Braulidae, Platystomatidae, Pyrgotidae (Diptera)	358
77.0	Agromyzidae (Diptera)	3017	Calyptratae (Diptera)	20979
40.2	Fergusoninidae (Diptera)	29	Periscelididae (Diptera)	91
102.3	Opomyzidae (Diptera)	61	Carnidae, Odiniidae (Diptera)	157
102.7	Cecidomyiidae (Diptera)	6296	Pleciidae, Sciaridae (Diptera)	2735
206.4	Boreidae (Mecoptera)	38	Siphonaptera	2078
302.4	Lepidoptera	151318	Trichoptera	14193
103.5	Cynipidae (Hymenoptera)	1000	Figitidae (Hymenoptera)	1500
154.2	Anthophila (Hymenoptera)	19904	Sphecidae (Hymenoptera)	724
416.6	Condylognatha (Hemiptera+ Thysanoptera)	85736	Psocodea	9234
292.2	Pentatomomorpha (Hemiptera)	13944	Cimicomorpha (Hemiptera)	7895
204.6	Thaumastocoridae (Hemiptera)	19	Anthocoridae, Cimicidae, Lyctocoridae, Plokiophilidae (Hemiptera)	733

Note- Clade A denotes the phytophagous group and Clade B its non- phytophagous sister clade. All richness estimates are given in terms of corrected richness; see text for discussion. Node ages are based on the consensus topology of Rainford et al. (2014).

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Tables

Table 1: Overall Likelihood and Transition rates per million years inferred for the optimal ML model of Larval Raw Data set.

	Fungivory	Detritivory	Phytophagy	Predators	Parasitoids	Ecto-parasites
Fungivory	NA	0.00187	0.00060	0.00065	0.00022	0.00
Detritivory	0.00016	NA	0.00039	0.00072	0.00016	0.00004
Phytophagy	0.00031	0.00015	NA	0.00003	0.00010	0.00
Predators	0.00007	0.00016	0.00015	NA	0.00009	0.00004
Parasitoids	0.00	0.00	0.00035	0.00016	NA	0.00011
Ecto-parasites	0.00	0.00	0.00	0.00	0.00	NA

Note- Overall LnL: 563.59, AIC: 1187.17, n. taxa: 874. Models are denoted as transition rates from rows to columns.

Table 2: Statistical tests of sister group comparisons.

Coding system	Ecology	Method	N cont.	N succ.	p value	Raw Contrasts		Log Ratio Contrasts		Proportional Contrasts	
						W (S*)	p (two tailed)	W (S*)	p (two tailed)	W (S*)	p (two tailed)
Larval (Raw)	Fungivory	P	15	5	0.3333	45	0.4212	50	0.5995	50	0.560
Larval (Raw)	Fungivory	ML	16	5	0. 3125	42	0.1928	49	0.3484	43	0.211
Larval (Raw)	Detritivory	P	19	4	0.2105	31	0.0082	54	0.1042	49	0.066
Larval (Raw)	Detritivory	ML:	24	13	0.5417	111	0.4202	134	0.9152	120	0.595
Larval (Raw)	Phytophagy	P	25	10	0.400	123	0.2940	127	0.3463	151	0.767
Larval (Raw)	Phytophagy	ML	-	-	-	-	-	-	-	-	-
Larval (Raw)	Predators	P	31	15	0.4839	232.0	0.7613	222	0.6173	222	0.617
Larval (Raw)	Predators	ML	29	13	0.4483	210	0.8797	215	0.9655	214	0.948
Larval (Raw)	Parasitoids	P	15	5	0.3333	45	0.4212	50	0.5995	50	0.600
Larval (Raw)	Parasitoids	ML	-	-	-	-	-	-	-	-	-
Larval (Modified)	Phytophagy	P	25	11	0.440	122	0.2818	127	0.3463	157	0.893
Larval (Modified)	Phytophagy	ML	26	12	0.4615	124	0.1952	144	0.4311	171	0.919
Larval (Modified)	Parasites	P	19	7	0.3684	78	0.5153	81	0.5949	81	0.595
Larval (Modified)	Parasites	ML	-	-	-	-	-	-	-	-	-
Adult	Fungivory	P	18	6	0.3333	52	0.1541	60	0.2837	66	0.417
Adult	Fungivory	ML	10	2	0.2	81492*	0.8320	9.672*	0.1211	1.685*	0.174
Adult	Phytophagy	P	12	7	0.5833	17	0.0923	33	0.6772	151	0.733
Adult	Phytophagy	ML	-	-	-	-	-	-	-	-	-
Adult	Predators	P	13	5	0.385	43	0.8926	43	0.8926	43	0.893
Adult	Predators	ML	12	5	0.417	35	0.7910	36	0.8501	35	0.791
Adult	Blood feeders	P	7	5	0.7143	4684*	0.9688	5.107*	0.2031	1.070*	0.266
Adult	Blood feeders	ML	5	2	0.400	11613*	0.8125	0.261*	1.0	0.068*	0.938

Note- Methods of reconstruction are denoted P; parsimony and ML; Maximum Likelihood. Number of contrasts (N. cont.) and number of successes (where the richness of the focal origination was greater than its sister clade ; N succ.) are denoted for interpreting the sign test. SDC methods are given as results of Wilcoxon tests or their normalized equivalent, with the exception of tests denoted by an asterix which are the results of randomized matched pairs.

Figure Legends

Figure 1: Indices of phylogenetic clustering for different character states inferred for the different coding systems. Plotted values are the observed Net Relatedness index (NRI) and Nearest Taxon index (NTI) for diets. NRI and NTI are dimensionless ratios defined as: the difference between the observed and mean expected values of: the total phylogenetic distance of taxa possessing a particular diet (NRI) and the mean distance between taxa with particular diets (NTI), divided by the standard deviation of expected values, based on 999 randomized tip permutations. Positive index values denote phylogenetically clustered data (Webb et al. 2008). The null interval, i.e. no significant deviation from zero (dashed line), is denoted by the dotted lines. Symbols used for datasets follow the internal legends. Values and associated test statistics are given in Table A1.

Figure 2: Reconstructed dietary ecologies for the Larval Raw dataset under maximum likelihood. Ecologies are denoted as follows: Dark Blue- Fungivory, Cyan- Detritivory, Green- Phytophagy, Red- Predatory, Magenta- Parasitoids, Yellow- Ectoparasites. Taxa and nodes with mixed states are shown by dashed lines. Taxa with unknown states are shown in Grey. Colored dots denote the postions of sister group comparisons (Table 2). The coloration of the outer ring denotes major clades (Grey; Entognatha, Black; basal insects, Cyan; Palaeoptera, Purple; Polyneoptera, Green; Paraneoptera, Red; Holometabola). Internal piechart gives the relative species richness associated with each dietary category, with taxa with mixed ecologies contributing to all relevant states, see Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v.

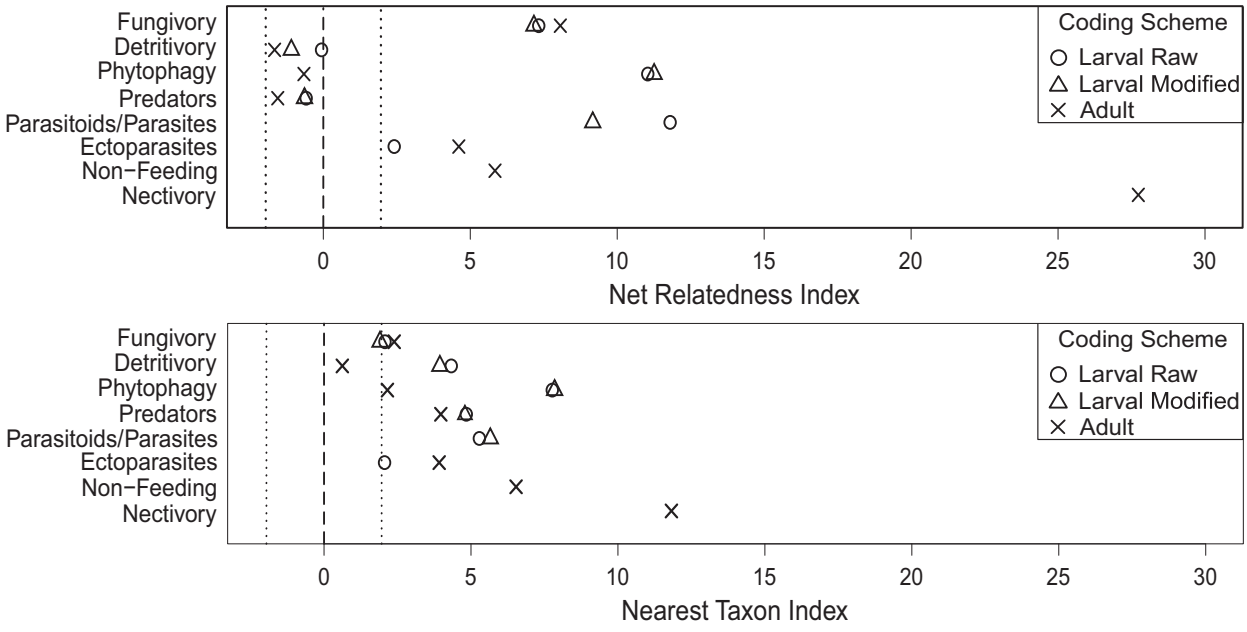
Figure 3: Accumulation Plot of dietary originations through geological time for Larval_Raw ML reconstruction.

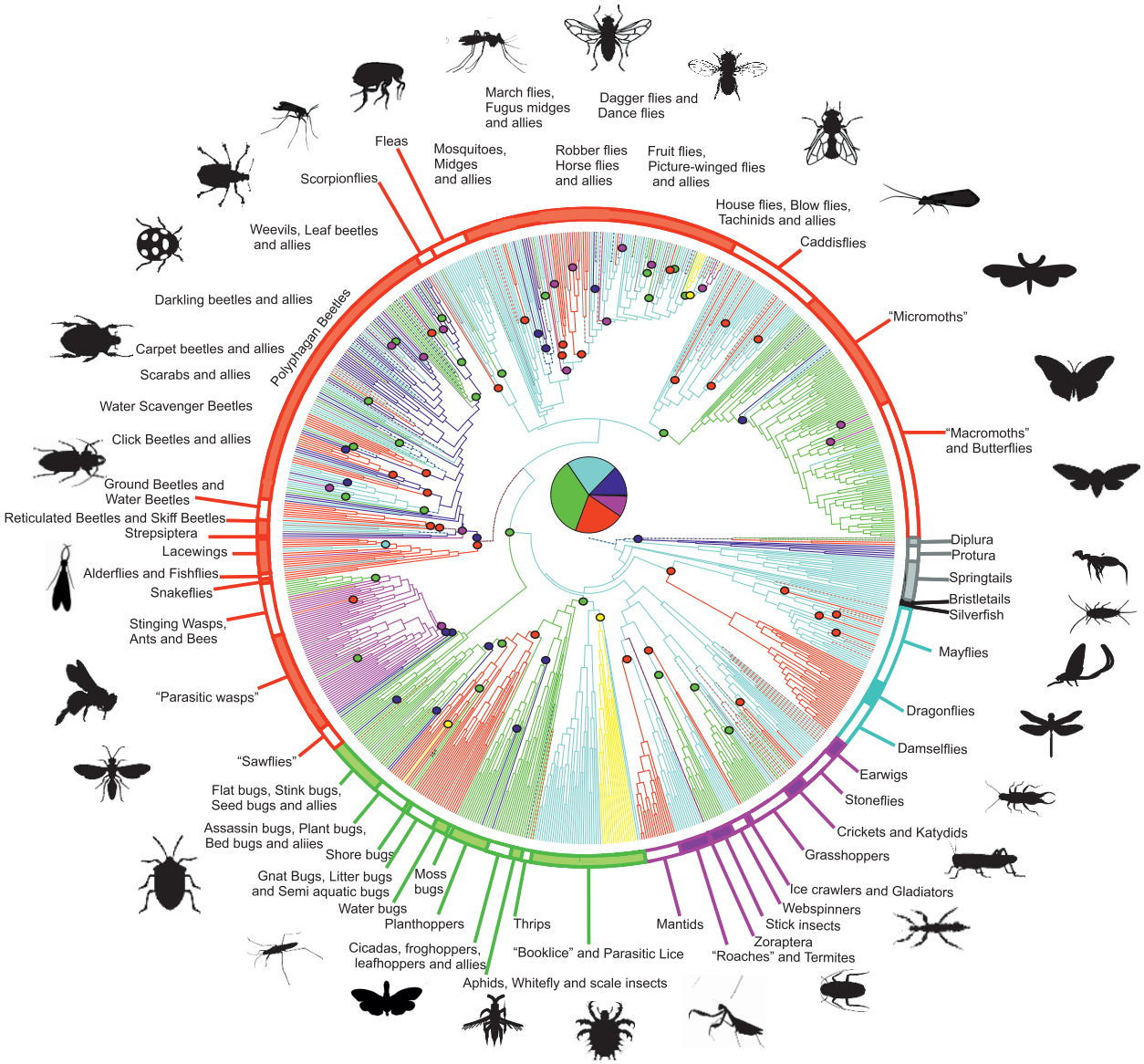
Figure 4: Reconstructed adult dietary ecology under maximum likelihood. Colors are as Figure 2 with the addition of Orange- NonFeeding and Pink- Liquid feeding/Nectivory. Yellow is used to denote both Ectoparasites and adult blood feeding taxa. Colored dots denote the positions of sister group comparisons (Table 2). Internal piechart gives the relative species richness associated with each dietary category with taxa with mixed ecologies contributing to all relevant states, see Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v.

Online Figure A1: Reconstructed ecologies for the Larval Modified data set under maximum likelihood. Colors are as Figure 2 with Magenta denoting carnivorous parasites. Internal piechart gives the relative species richness associated with each dietary category with taxa with mixed ecologies contributing to all relevant states see Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6f75v.

Online Figure A2: The reconstructed history of the "potential specialization" (PS) character described in the text. Clades with specialized ecologies are denoted in red, generalized ecologies in blue and taxa with unknown states in grey. Mixed states are shown by dashed lines. Tree orientation and clade identities are as Figure 2.

Figure PDF for Production

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Dagger flies and
Dance flies
Fleas
Mosquitoes,
Midges
and allies
Robber flies
Horse flies
and allies
Fruit flies,
Picture-winged flies
and allies
Scorpionflies
House flies, Blow flies,
Tachinids and allies
Weevils, Leaf beetles
and allies
Caddisflies
Darkling beetles and allies
"Micromoths"
Polyphagan Beetles
Carpet beetles and allies
Scarabs and allies
"Macromoths"
and Butterflies
Water Scavenger Beetles
Click Beetles and allies
Diplura
Protura
Springtails
Ground Beetles and
Water Beetles
Bristletails
Silverfish
Reticulated Beetles and Skiff Beetles
Strepsiptera
Mayflies
Lacewings
Alderflies and Fishflies
Dragonflies
Snakeflies
Damselflies
Stinging Wasps,
Ants and Bees
Earwigs
"Parasitic wasps"
Stoneflies
"Sawflies"
Crickets and Katydids
Flat bugs, Stink bugs,
Seed bugs and allies
Grasshoppers
Assassin bugs, Plant bugs,
Bed bugs and aliies
Ice crawlers and Gladiators
Shore bugs
Webspinners
Gnat Bugs, Litter bugs
and Semi aquatic bugs
Moss
bugs
Stick insects
Water bugs
Zoraptera
Planthoppers
Thrips
Mantids
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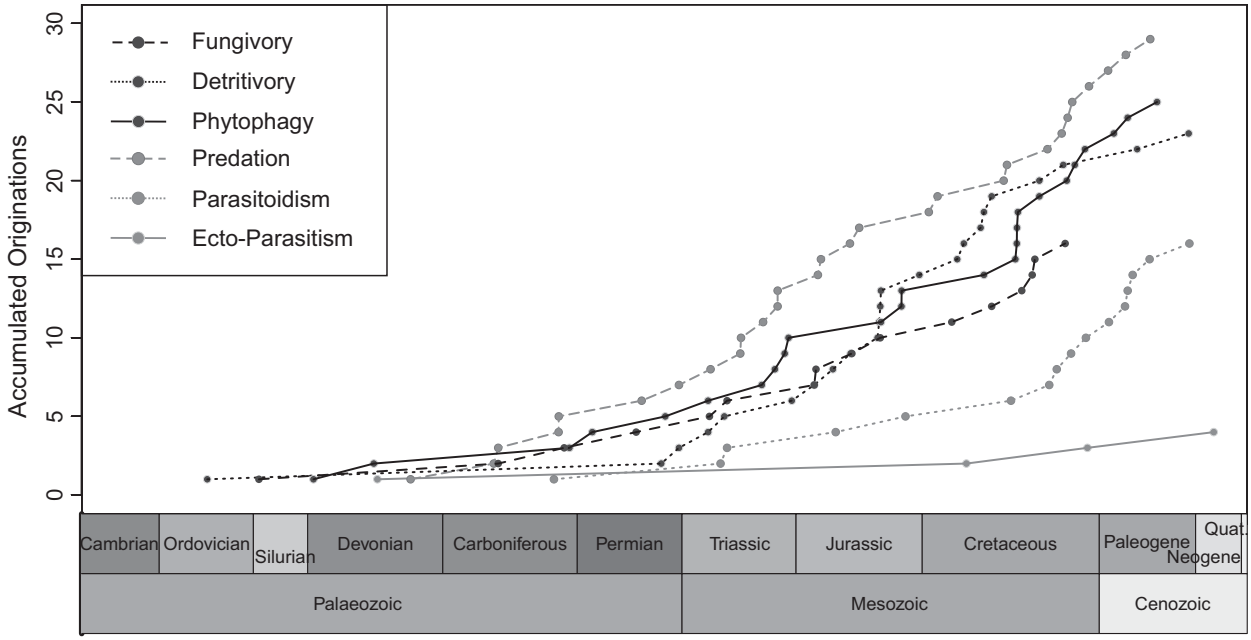
Cicadas, froghoppers,
leafhoppers and allies
"Booklice" and Parasitic Lice
Aphids, Whitefly and scale insects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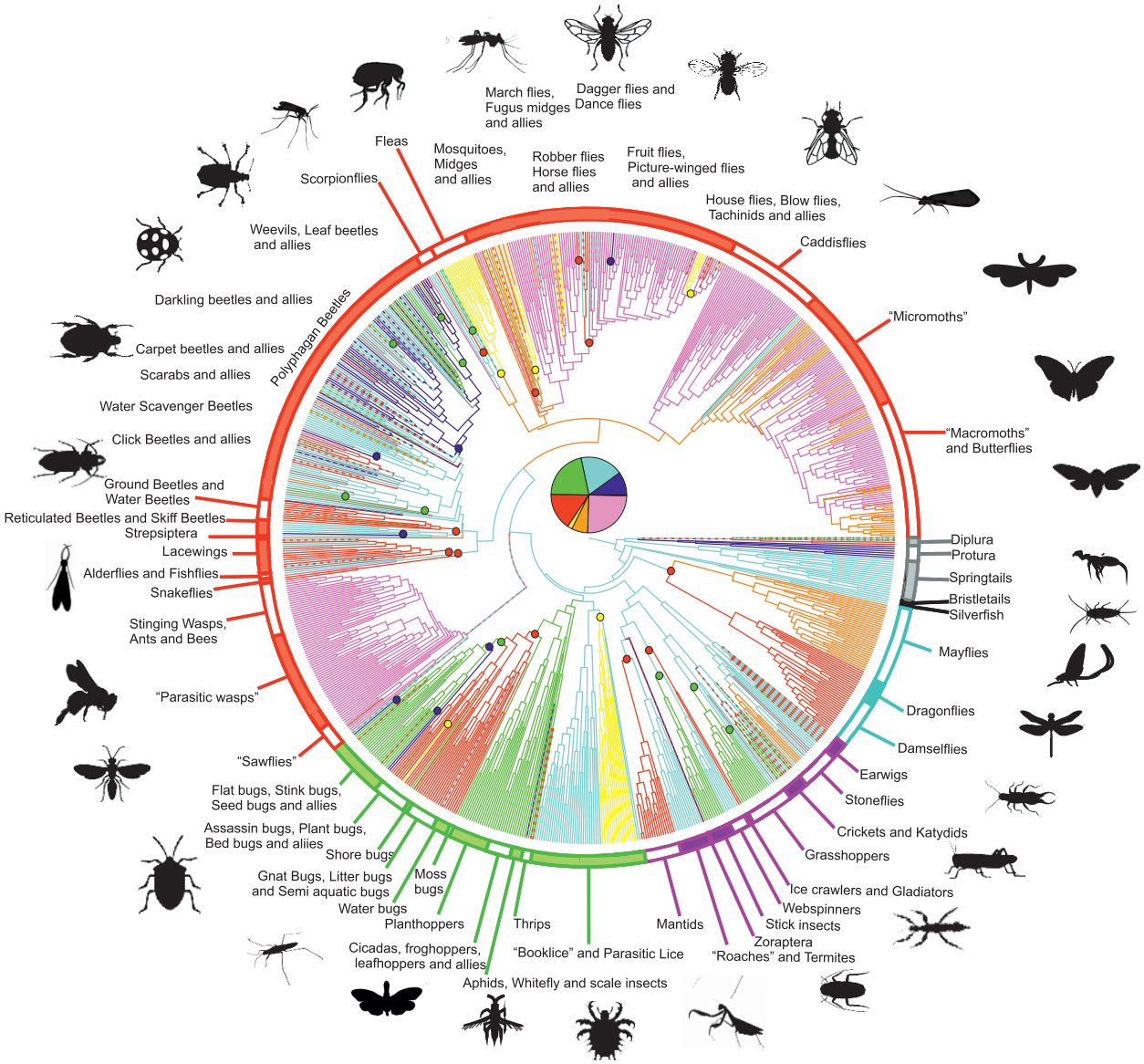
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Bristletails
Silverfish
Reticulated Beetles and Skiff Beetles
Strepsiptera
Mayflies
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Alderflies and Fishflies
Dragonflies
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Grasshoppers
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Shore bugs
Webspinners
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and Semi aquatic bugs
Moss
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Stick insects
Water bugs
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leafhoppers and allies
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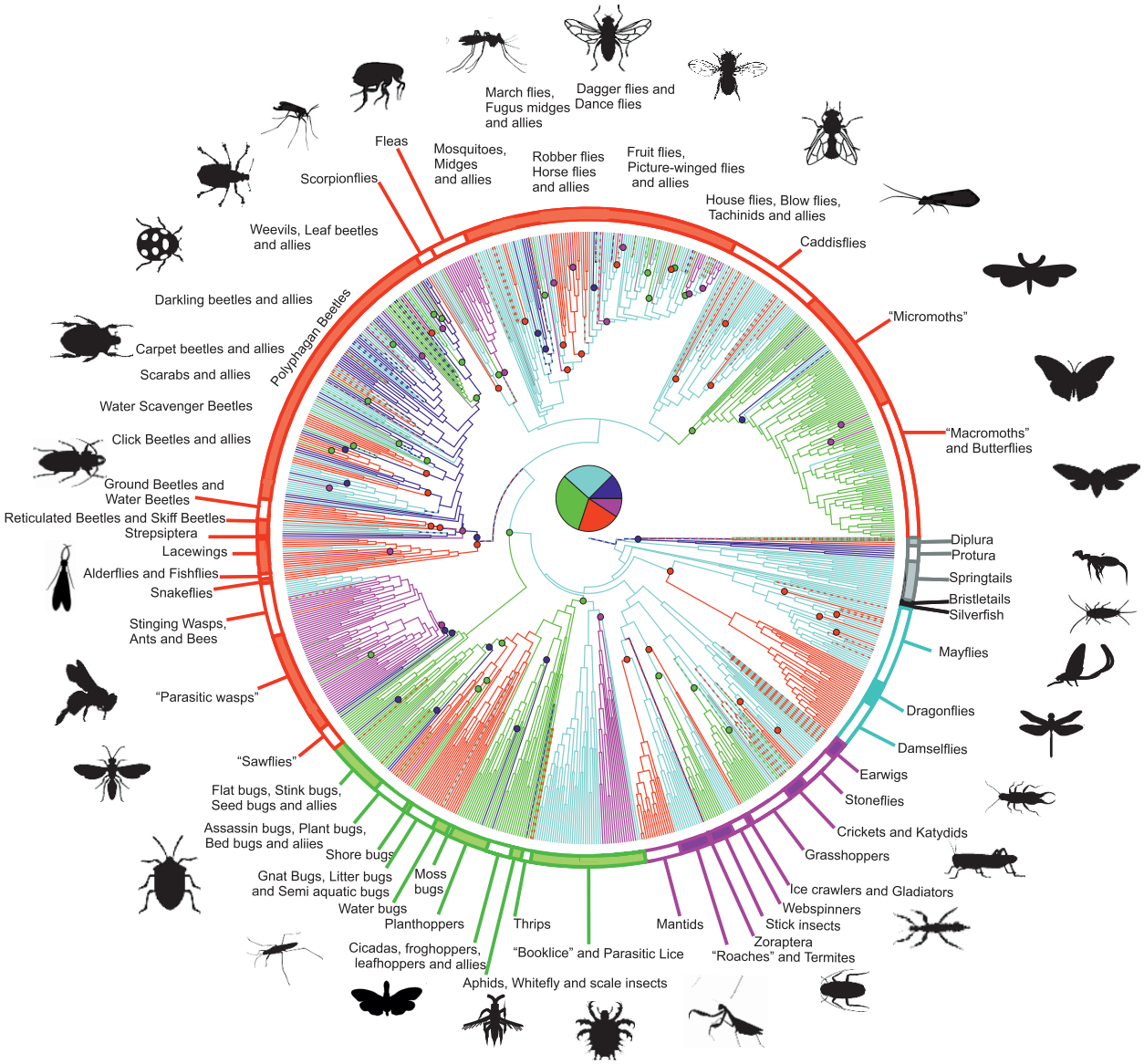
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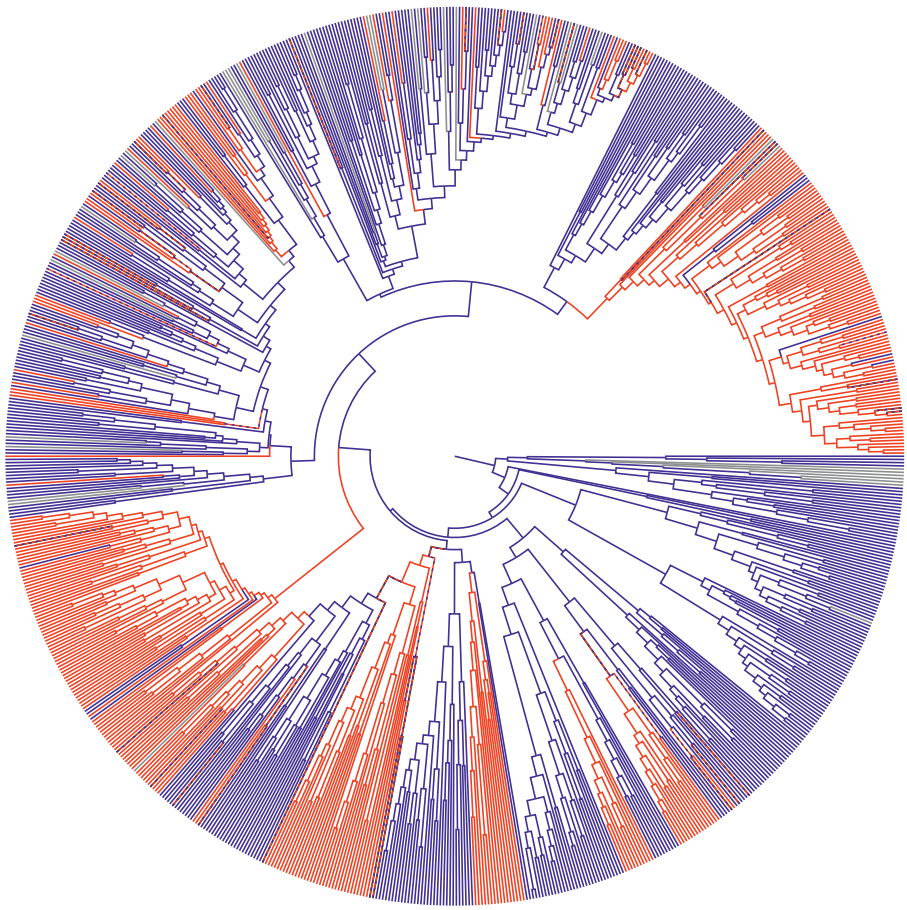

Figure 1

Figure 2

Figure 3

Figure 4

Figure A1

Figure A2