Bridging the Gap across Micro-, Macro- and Mega-Evolutionary Timescales

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Abstract

In this thesis I explore evolution spanning many orders of magnitude, from micro- to macro- and mega- evolutionary timescales. In the first part of this thesis I investigate the connection between micro- and macro- evolution in the morphological traits of organisms. I aim to understand why phenotypic evolution is conservative over short timescales yet net change accumulates over longer timescales via macroevolutionary bursts. I find that a simple process-based model of microevolution playing out over rugged adaptive landscapes can account for the empirical pattern of divergence in animal body sizes over time and that macroevolutionary bursts can be explained by rare traversals of valleys in the adaptive landscape without invoking shifts in adaptive peaks themselves. Secondly, I explore the connection between the macroevolutionary rate of speciation and the rates of steps involved in the speciation cycle, encompassing the evolution of geographical, reproductive and ecological isolation between populations. I show via a mathematical model that this relationship is nonlinear so that macroevolutionary and microevolutionary rates may appear disconnected for certain steps of the speciation cycle. Thirdly, I investigate the macro and megaevolutionary dynamics of the evolution of bill size in relation to body size across the entire radiation of modern birds. I find that evolution is generally allometrically conservative over millions of years, but there have been multiple shifts in the slope and intercept of the allometric relationship between bill size and body size across different clades accumulating steadily over time. Finally, I turn my attention to a specific case study in macroevolution, hummingbird diversification and morphological evolution in relation to the evolution of foraging ecology. I test the hypothesis that diversification rate is associated with foraging ecology, specifically that a traplining ecology which is thought to entail ecological specialisation is an ‘evolutionary dead end’. I find no evidence for a relationship between foraging ecology and rates of diversification. Taken together, this thesis finds that the tempo and mode of evolution are heterogeneous over timescales. Nonetheless, our understanding of evolutionary patterns over different timescales can be bridged by understanding how underlying processes interact and scale over time.

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I am also very grateful to my parents for instilling an early love of nature in me and for supporting me throughout my studies, even when times were tough.

Declaration

I confirm that the work submitted in this thesis is my own, that I am the primary author, and corresponding author for all publications arising from this work. I include the following acknowledgments, and explicitly indicate contributions for jointly authored publications. I confirm that appropriate credit has also been given within the thesis where reference has been made to the work of others.

Chapter 4, **Allometric Conservatism in the Evolution of Bird Beaks**, is published in its presented form in **Evolution Letters** (2022). I and my lead supervisor Gavin Thomas conceived and designed the study. Eliott Capp, Chris Cooney, Zoe Varley and Emma Hughes collected the data. I analysed the data and drafted the manuscript. All authors gave final approval for publication of the manuscript.

Chapter 5, **The Evolution of the Traplining Pollinator Role in Hummingbirds: Specialisation is not an Evolutionary Dead End**, is published in its presented form in **Proceedings of the Royal Society B** (2022). I and my supervisors Gavin Thomas, Andrew Beckerman and Natalie Cooper conceived and designed the study. Eliott Capp, Zoe Varley and Emma Hughes collected the data. I analysed the data and drafted the manuscript. All authors gave final approval for publication of the manuscript.

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Chapter 1
General Introduction

The modern theory of evolution provides a set of first principles for understanding and predicting a wide range of evolutionary phenomena, from the sex ratio expected in the alates of an ant colony, the incidence of helping at the nest in long-tailed tits and the showy displays of male peacocks to character displacement between species of Galapagos finches and their adaptive radiation on an archipelago free of competitors. The theory has been quite successful, which is not to say that there aren't still some sticking points. One of these is the question of how to reconcile the tempo and mode of evolution over different timescales. If the relevant parameters are known, the theory can make quite precise predictions about evolutionary change in the short term, a snapshot of 'microevolution'. The Price (1970) equation and its derivatives can predict the expected course of evolution given knowledge of selection and the heritability of traits. Over more than a few generations the parameters governing evolution are liable to change and predictions become increasingly probabilistic. This then blends into 'macroevolution'. Macroevolution encompasses changes over longer timescales that are typically associated with divergence between species. 'Megaevolution' is a term coined by Simpson (1944; 1953) to refer to evolution over even longer timescales that are typically associated with divergence between higher taxonomic units such as families, orders and phyla. How are these different timescales of evolutionary change related?

While microevolution in quantitative traits can be precisely predicted by the breeder's equation over a few generations, models with a higher level of abstraction are necessary to describe evolution over longer timescales. The two most prominent models of macroevolution in phenotypic traits are the Brownian Motion (BM) model (Lande, 1976) and the Ornstein-Uhlenbeck (OU) model (Hansen & Martins, 1996; Hansen, 1997; Butler & King, 2004). Both of these models feature several times in the following chapters of this thesis. The BM model assumes that the sum of evolutionary pressures acting on a population over time are independent and normally distributed so that the mean phenotype of a population wanders randomly through the space of possible phenotypes. It predicts that the divergence between independently evolving populations or species should grow linearly with time. The OU model on the other hand assumes that evolutionary pressures have a central tendency towards an 'optimum' around which there are random fluctuations. It predicts that an equilibrium variance around the evolutionary optimum should emerge among independently evolving populations or species. There are many extensions of these basic models. For instance, across the branches of the tree of Life the rate of evolutionary change in the BM model may vary (Venditti et al., 2011), and in the OU model the position of the optimum may vary (Uyeda & Harmon, 2014). Shifts between evolutionary regimes among clades extend the BM and OU models of macroevolution to the level of megaevolution. In chapter 4 I use a multi-regime OU model to study megaevolutionary shifts in the scaling relationship between beak size and body size in birds. In chapter 5 I apply a BM model with multiple evolutionary regimes associated with three discrete foraging ecologies mapped to a phylogeny of hummingbirds.

The BM and OU models by themselves are purely phenomenological. However, they can be reconciled with explicit microevolutionary processes (Lande, 1976; 1985; Hansen & Martins, 1996) (fig. 1.1). In the BM model the evolutionary rate parameter can be expressed in terms of basic quantitative genetic parameters of a population. One such parameterisation assumes that the trait is selectively neutral and evolves under mutation-drift balance alone. However, selection can be incorporated into the model as well. For one, it could be assumed that directional selection pressures fluctuate randomly and independently in successive time intervals. Yet another parameterisation incorporating selection assumes that there is relatively strong stabilising selection towards fitness peaks, but the positions of these peaks fluctuate randomly over time. There are also various microevolutionary interpretations of OU evolution. One interpretation is that the course of evolution is subject to random genetic drift as well as stabilising selection towards a stationary fitness peak. Another interpretation incorporates fluctuating directional selection pressures that have a central tendency over time. There is a many-to-one mapping between underlying evolutionary processes and the evolutionary patterns predicted by the BM and OU models.

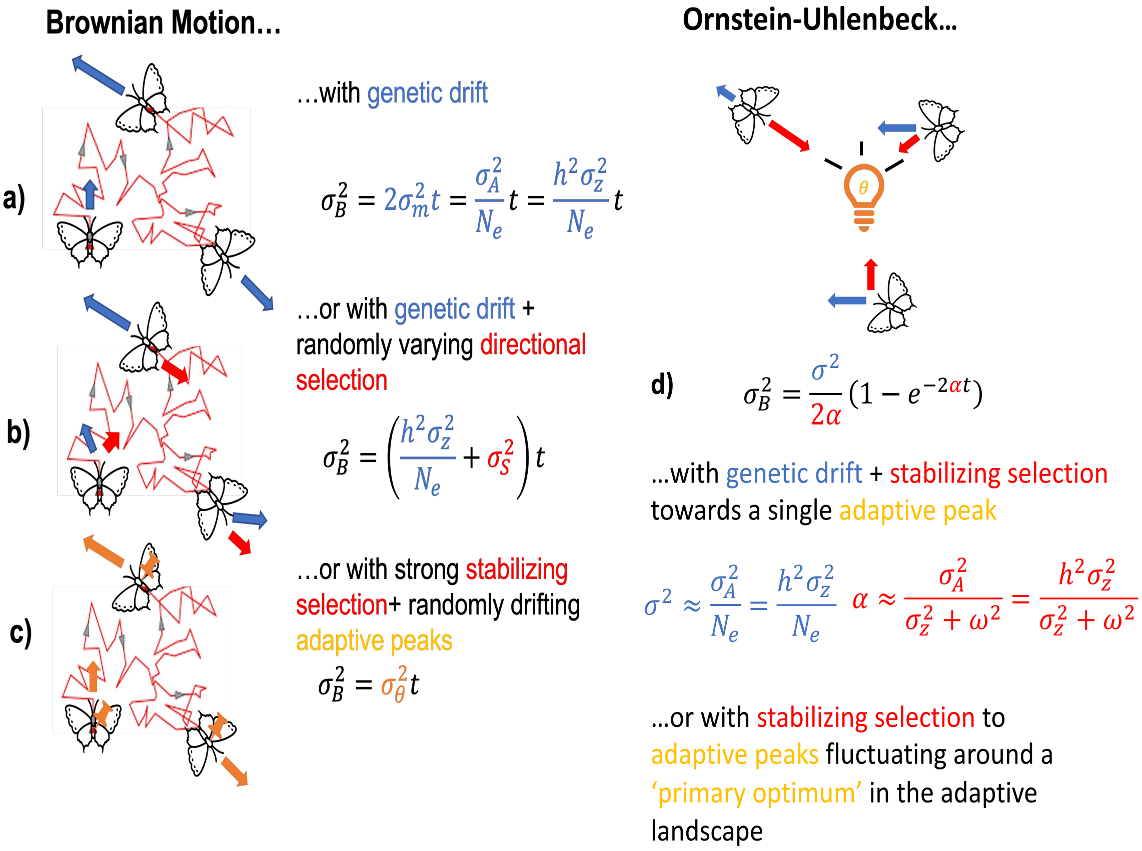

Fig 1.1. Relating microevolutionary processes to macroevolutionary models of phenotypic evolution, where σ_B^2 is the growing variance between divergently evolving populations or species and t is time. Other parameters correspond to various quantitative genetics features of populations.

One pattern that the BM and OU models do not predict is a mode of evolution with long periods of relative stasis interrupted by relatively rapid bursts of evolutionary change. This pattern was suggested to be prevalent in the fossil record (Eldredge & Gould, 1972), where an apparent absence of transitional forms may not necessarily be an artefact of imperfect preservation but a reflection of rapid bursts of evolution too short in duration to be preserved. The term ‘punctuated equilibrium’ was coined for this pattern, immediately becoming quite controversial. It does not help that after this original paper introducing punctuated equilibrium was published, several separate ideas in the paper became unnecessarily conflated under that same term (Pennell, Harmon & Uyeda, 2014). These are: the suggestion that phenotypic evolution tends to be pulsed rather than gradual, that these pulses are associated with speciation events, and that the differential diversification of lineages with different traits leads to macroevolutionary trends. There is increasing evidence that pulsed evolution is a common pattern in the evolution of Life (Uyeda et al., 2011; Landis & Schraiber, 2017; Mattila & Bokma, 2008). If pulsed evolution is a common pattern, what are the processes generating it? This question is taken up in chapter 2 of this thesis where two potential hypotheses for the existence of the pattern are contrasted and pitted against empirical data.

Phenotypic evolution is only one dimension of the process whereby Life diversifies. Speciation is the other dimension. Here too we can ask the question of how our understanding of microevolutionary processes translates into macroevolutionary patterns. A cross-section of all Life at the present reveals that many species consist of populations at different stages of diverging from each other. To become independently evolving lineages in the long term, with the species concept of Barraclough (2019) in mind, incipiently diverging population must first acquire geographical, reproductive and ecological isolation in most cases. It would make sense to predict that the rates of accrual of geographical, reproductive and ecological isolation should correlate with macroevolutionary rates of speciation. Surprisingly, this seems often not to be the case (Rabosky & Matute, 2013; Singhal et al., 2022; 2018). Chapter 3 explores a general explanation for this based on considering a model of protracted speciation in which speciation is modelled as a process consisting of several successive steps. An interesting idea linking phenotypic evolution and speciation is that differential rates of speciation and extinction could be associated with the evolution of certain traits, leading to biased representation of certain phenotypes as time goes on. This idea has come to be known as species selection (Jablonski, 2008). Chapter 5 of this thesis examines whether foraging traits of hummingbirds are associated with differential rates of diversification and whether a particular foraging ecology is an 'evolutionary dead end'.

The following is an outline of the questions explored in this thesis and the methods used to address them. Firstly, how do the tempo and mode of evolution translate from microevolutionary to macroevolutionary timescales? Are there common underlying processes at play over all timescales or are there special processes that need to be invoked to account for macroevolutionary patterns? Hence, is macroevolution predictable on the basis of microevolution? Chapter 2 of this thesis examines these questions. I use Approximate Bayesian Computation (ABC) to fit two alternative process-based models of evolution to empirical data on phenotypic divergence in the body size of animals over different timescales. Secondly, what is the relationship between macroevolutionary rates of speciation and the rates of incremental accrual of geographical, reproductive and ecological isolation between incipiently diverging species? Chapter 3 of this thesis takes a theoretical approach to this question to examine what the general relationship would be between macroevolutionary and microevolutionary rates on the basis of a model of protracted speciation. Thirdly, what patterns emerge across macro- to mega- evolutionary timescales? In chapter 4 I explore the megaevolutionary dynamics of the allometric relationship between beak size and body size across the history of all living birds. Finally, in chapter 5 I ask the question whether different rates of diversification associated with different foraging ecologies has shaped hummingbird evolution.

References

1. Barraclough (2019). The evolutionary biology of species. Oxford University Press
2. Butler MA & King AA (2004). Phylogenetic comparative analysis: a modelling approach for adaptive evolution. *American Naturalist.* 164(6):
3. Eldredge, N., & Jay Gould, S. (1972). Punctuated equilibria: an alternative to phyletic gradualism. In *Models in Palaeobiology* (pp. 82–115).
4. Hansen TF (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution. 51(5):* 1341-1696
5. Hansen, T. F., & Martins, E. P. (1996). Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution*, *50*(4), 1404–1417.
6. Jablonski, D. (2008). Species selection: Theory and data. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 501–524. https://doi.org/10.1146/annurev.ecolsys.39.110707.173510
7. Lande, R. (1976). Natural Selection and Random Genetic Drift in Phenotypic Evolution. *Evolution*, *30*(2), 314. https://doi.org/10.2307/2407703
8. Lande, R. (1985). *Expected time for random genetic drift of a population between stable phenotypic. 82. PNAS*
9. Landis, M. J., & Schraiber, J. G. (2017). Pulsed evolution shaped modern vertebrate body sizes. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(50), 13224–13229. https://doi.org/10.1073/pnas.1710920114
10. Mattila, T. M., & Bokma, F. (2008). Extant mammal body masses suggest punctuated equilibrium. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1648), 2195–2199. https://doi.org/10.1098/rspb.2008.0354
11. Pennell, M. W., Harmon, L. J., & Uyeda, J. C. (2014). Is there room for punctuated equilibrium in macroevolution? In *Trends in Ecology and Evolution* (Vol. 29, Issue 1, pp. 23–32). https://doi.org/10.1016/j.tree.2013.07.004
12. Rabosky, D. L., & Matute, D. R. (2013). Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in Drosophila and birds. *PNAS*, *110*(38), 15354–15359. https://doi.org/10.1073/pnas.1305529110
13. Price GR (1970). Selection and covariance. *Nature.* 227: 520-521
14. Simpson, G. (1944). *Tempo and Mode in Evolution*.
15. Simpson, G. (1953). *The major features of evolution*.
16. Singhal, S., Colli, G. R., Grundler, M. R., Costa, G. C., Prates, I., & Rabosky, D. L. (2022). No link between population isolation and speciation rate in squamate reptiles. *PNAS*, *119*(4). https://doi.org/10.1073/pnas.2113388119/-/DCSupplemental
17. Singhal, S., Huang, H., Grundler, M. R., Marchán-Rivadeneira, M. R., Holmes, I., Title, P. O., Donnellan, S. C., & Rabosky, D. L. (2018). *Does Population Structure Predict the Rate of Speciation? A Comparative Test across Australia's Most Diverse Vertebrate Radiation*. https://doi.org/10.5061/dryad.j6823nt

18. Uyeda, J. C., Hansen, T. F., Arnold, S. J., & Pienaar, J. (2011). The million-year wait for macroevolutionary bursts. *Proceedings of the National Academy of Sciences*, *108*(38), 15908–15913. https://doi.org/10.1073/pnas.1014503108
19. Uyeda, J. C., & Harmon, L. J. (2014). A novel Bayesian method for inferring and interpreting the dynamics of adaptive landscapes from phylogenetic comparative data. *Systematic Biology*, *63*(6), 902–918. https://doi.org/10.1093/sysbio/syu057
20. Venditti, C., Meade, A., & Pagel, M. (2011). Multiple routes to mammalian diversity. *Nature*, *479*(7373), 393–396. https://doi.org/10.1038/nature10516

Chapter 2
The Evolutionary Continuum Hypothesis: Adaptive Landscapes Bridge the Gap Between Micro- and Macro- Evolution

2.1 Abstract

How do we reconcile macroevolutionary patterns with microevolutionary processes? Evolution in traits such as body size tends to be characterised by bounded fluctuations around a mean from a few generations up to a million years or more. Previous work has shown that it takes on the order of a million years on average between macroevolutionary bursts of body size evolution. This pattern needs a process-based explanation. One potential hypothesis invokes rare shifts in the location of adaptive peaks themselves. Here I consider a previously suggested alternative hypothesis that explains this pattern as an emergent consequence of lineages traversing a rugged adaptive landscape, invoking no discontinuities between processes operating over micro- and macro- evolutionary timescales. This is the evolutionary continuum hypothesis. Using Approximate Bayesian Computation (ABC) I show that a simple 3-parameter process-based model inspired by this hypothesis can fit the observed pattern of divergence in animal body sizes over time equally well as existing phenomenological models with burst-like dynamics. A testable implication of the evolutionary continuum hypothesis is that certain aspects of microevolution could predict rates of macroevolution.

2.2 Introduction

The course of evolution does not always run smooth. Simpson (1944; 1953) envisioned that evolution typically follows a pattern of relative conservatism as lineages explore the space of possible phenotypes within an adaptive zone, broken by occasional shifts to new adaptive zones in different regions of the adaptive landscape. Indeed, evolution in both shape and size traits has been found to be characterised by bounded fluctuations around a mean from a few generations up to a million years or more, even as the potential for more rapid directional evolution exists in most animal populations (Estes & Arnold, 2007). This relative conservatism is broken over longer timescales of millions of years by bursts of evolutionary change (Uyeda et al., 2011; Landis & Schraiber, 2017; Mattila & Bokma, 2008). What processes cause this evolutionary pattern?

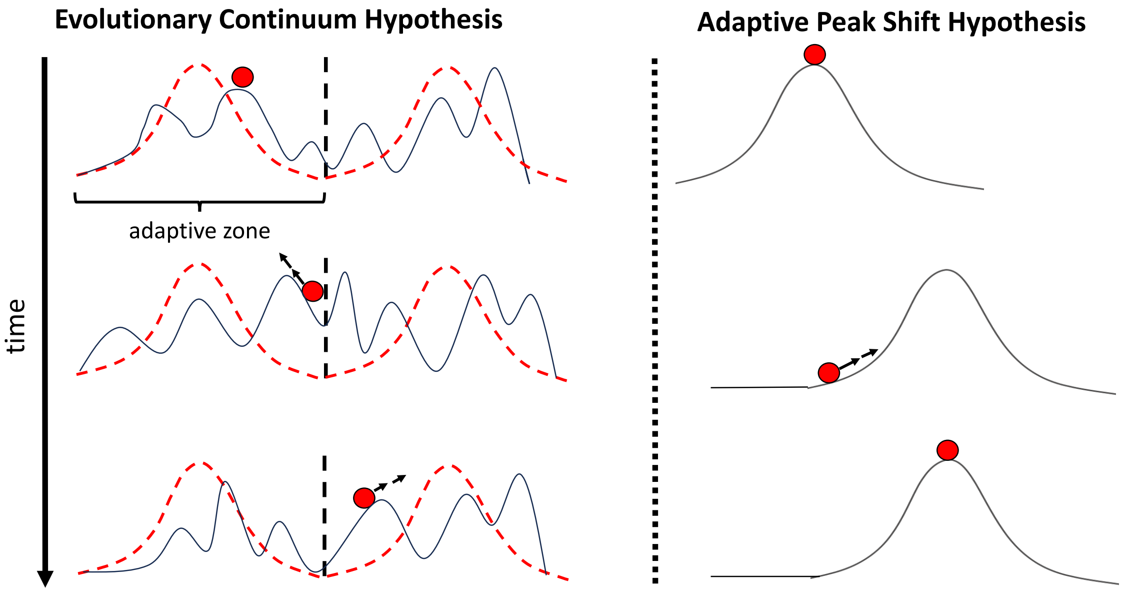

Fig 2.1. Contrasting the adaptive peak shift hypothesis with the evolutionary continuum hypothesis. In the former (righthand panel), bursts of evolution are driven by relatively abrupt shifts in the locations of adaptive peaks themselves. In the latter (lefthand panel), bursts of evolution are an emergent consequence of lineages traversing a rugged adaptive landscape. The red dashed curves are the adaptive peaks within each adaptive zone, a distribution showing where fitness peaks represented by the solid black curves are most likely to be found over time. Fluctuations within adaptive zones are due to a combination of fluctuating selection pressures as well as genetic drift. Note that the middle panel on the left is an unlikely configuration of the fitness peaks within an adaptive zone, and even then the process of crossing the threshold to the neighbouring adaptive zone requires genetic drift into a fitness valley.

One potential hypothesis is that macroevolutionary bursts of evolution are caused by shifts in the locations adaptive peaks themselves (fig. 2.1 right panel), disconnected from the typical continuous fluctuations on the adaptive landscape occurring over microevolutionary timescales. Relatively rapid shifts in the locations of adaptive peaks may be triggered by events such as the colonisation of islands where there is ecological opportunity to adapt to new niches (Lovette et al., 2002), the evolution of key innovations (Simpson, 1953) such as the decoupling of oral and pharyngeal jaws in cichlid fishes (Liem, 1973), rapid cycles of Red Queen coevolution (Van Valen, 1973) such as the arms race between cuckoo-finches and their hosts (Dixit et al., 2023), or relatively abrupt environmental change over geological timescales such as the onset of an interglacial. Phenomenological models of evolution that include bursts typically model these bursts as effectively discrete shifts, either in phenotypes directly or in the adaptive peaks towards which phenotypes evolve (Uyeda et al., 2011; Landis & Schraiber, 2017; Mattila & Bokma, 2008; Uyeda & Harmon, 2014). An exception is the phenomenological model of Pagel, O'Donovan & Meade (2022) which treats evolutionary bursts in some lineages as random walks with a directional bias.

There exists an alternative hypothesis to explain evolutionary bursts against a background of relative conservatism, grounded in the emergent dynamics of evolution on rugged adaptive landscapes (Newman, Cohen & Kipnis, 1985; Lande, 1985). Rugged adaptive landscapes have multiple fitness peaks that could be occupied by a population separated by valleys of low fitness. Originally, this argument was framed mathematically in the context of microevolutionary fluctuations due to genetic drift around static fitness peaks. On a fitness landscape with two peaks, it is possible for a population to eventually cross from one peak to the other by genetic drift if the population is sufficiently small and the adaptive valley sufficiently shallow. Making it down into a fitness valley is an unlikely process taking a long time to happen by chance. Evolution from a fitness valley towards a neighbouring peak is fast given that the uphill evolution is promoted by natural selection. The population thus spends most of its time evolving around either fitness peak with little time spent in the fitness valley, so that the net result is a macroevolutionary burst when a lineage does eventually cross from one peak to the other in the course of its history.

This argument can be extended to fitness landscapes that fluctuate over time, so that the microevolutionary fluctuations involved in crossing from one adaptive zone to another are not solely reliant on genetic drift (fig. 2.1 left panel). Fluctuating selection is a much more efficient means of crossing from one adaptive zone into another, or at least to bring neighbouring peaks sufficiently close together that genetic drift has a chance to drive a population across a fitness valley. The macroevolutionary adaptive landscape has different interpretations depending on whether the underlying fitness landscape is assumed to be static or dynamic. If it is static, then fitness peaks correspond directly to adaptive peaks and fluctuations around them are purely due to genetic drift counteracting stabilising selection. If it is dynamic, adaptive peaks correspond to points in phenotype space where continuously moving fitness peaks are most likely to be found over time. In this case, fluctuations are due to a combination of fluctuating selection pressures as well as genetic drift. Lande (1976) showed that in the static case evolution follows an Ornstein-Uhlenbeck process (OU) with parameters σ^2 (the diffusion parameter causing divergence over time) and α (the conservative parameter pulling populations towards an optimum) that can be interpreted directly in terms of basic quantitative genetic parameters of a population including the additive genetic variance, the effective population size, and the strength of stabilising selection. It was later pointed out by Hansen & Martins (1996) that in the case of a dynamic fitness landscape evolution may still be approximated by an Ornstein-Uhlenbeck process if the sum of fluctuating selection pressures have a central tendency. Drifting fitness peaks in this scenario are assumed to concentrate around the centre of adaptive zones and only rarely venture towards the threshold to neighbouring adaptive zones.

Here I will use the Ornstein-Uhlenbeck process to model relatively conservative evolution around adaptive peaks, encompassing both dynamics driven by genetic drift around static fitness peaks as well as fluctuating selection pressures as fitness peaks drift around within adaptive zones. I will assume that adaptive peaks are distributed randomly and independently on the adaptive landscape as a Poisson process with a density of λ. By combining these two assumptions to create a generative model simulating the evolution of lineages on rugged adaptive landscapes, I will show that this process-based model with three parameters $(\sigma^2, \alpha, \lambda)$ is sufficient to explain the empirical pattern of phenotypic divergence in animal body sizes over time without invoking rare adaptive peak shifts to account for macroevolutionary bursts.

2.3 Methods

2.3.1 Simulation Algorithms

The algorithm for generatively simulating evolution under the evolutionary continuum hypothesis (ECH) is as follows. The phenotypic mean of a lineage starts at a randomly chosen position within its current adaptive zone on a phenotypic axis representing a single univariate trait such as body size. To the left and right of this adaptive zone are neighbouring adaptive zones. I assume for simplicity that all adaptive peaks are of the same height. Since adaptive peaks are assumed to be Poisson distributed on the adaptive landscape, the distances to the left and right neighbouring peaks are exponentially distributed. Evolution within adaptive zones is modelled as an OU process. Whenever a lineage gets closer to a neighbouring peak than it is to its current peak, the OU optimum parameter switches over to the neighbouring peak and new left and right neighbouring peaks are drawn from an exponential distribution after the lineage has had time to evolve towards its new adaptive peak. Before new left and right neighbours are drawn, there is the possibility that the lineage reverts to its old adaptive peak. The resolution of the main simulation is in increments of 10,000 years. To simulate OU evolution, I use the function r_ou in the R package goffda v 0.1.0 (Garcia-Portugues & Alvarez-Liebana, 2021). One example of such a simulation is shown in figure 2.2.

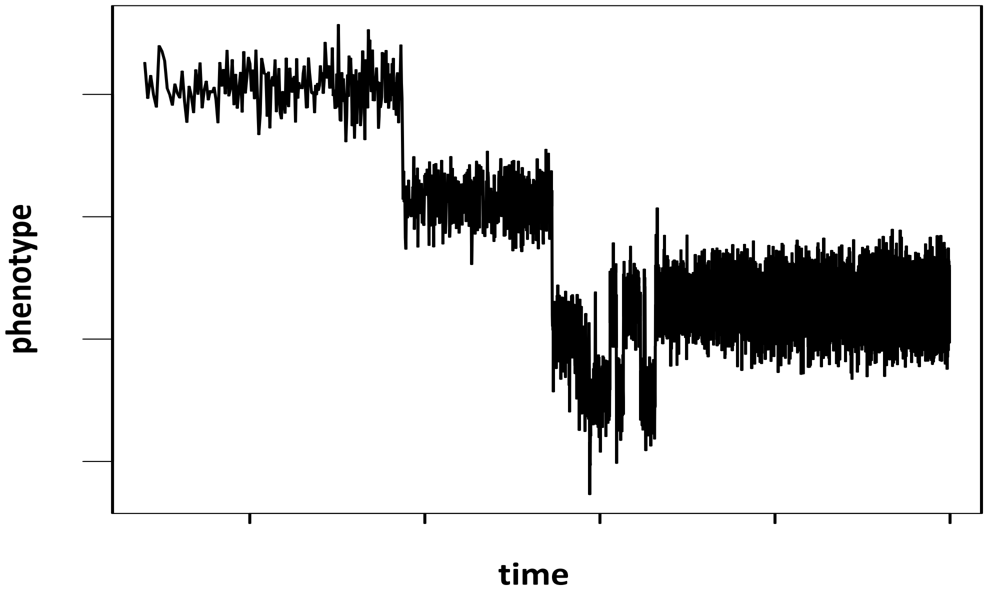

Fig 2.2. Phenotypic evolution under the ECH model showing general conservatism of OU evolution around adaptive peaks interspersed with macroevolutionary bursts when the population crosses the threshold between adaptive zones.

I contrasted this with another generative model simulating evolution under the adaptive peak shift hypothesis (APS). As with the model above, evolution around adaptive peaks is simulated as an OU process. In this model peak shifts are Poisson distributed through time, so the time to the next peak shift is drawn from an exponential distribution. The distance that the peak shifts either to the left or right along the phenotypic axis is taken to be another exponential distribution as in the ECH model. In this respect it differs from the model considered by Uyeda et al. (2011) where it was taken to be a normal distribution. A hundred independent simulations of evolution were run for both the ECH and APS models to obtain simulated vectors of variance in phenotypic divergence over time. The R code used to simulate evolution under both the ECH and APS models can be found at github: LRombaut/EvolutionaryContinuum.

It can be seen how these two process-based models are both connected to the patterns predicted by several existing phenomenological models of evolution (Uyeda et al., 2011; Landis & Schraiber, 2017). In the ECH model, the point at which a lineage crosses the threshold from one adaptive zone to the other is a rare event and the time to its occurrence should be approximately exponentially distributed, just as in the APS model. Where the two models differ however is that in the ECH model the distance between peaks should be correlated with the time to wait until a transition from one to the other occurs, whereas no such correlation between time and distance is built into the APS model. Both models imply that phenotypes after the first peak shift should be Laplace distributed, due to the exponential distributions of distances to the left and right neighbouring adaptive peaks in the ECH model or shift locations in the APS model. A Laplace shift process will lead to a variance-gamma distribution of phenotypes, differing from a normal distribution in having positive kurtosis or 'fat tails'. This predicts more evolutionary outliers than expected from a Brownian Motion process. By the central limit theorem however, the sum of many Laplace shifts will converge to a normal distribution with a variance growing roughly linearly over time as shifts accumulate stochastically, hence producing the same pattern predicted by Brownian Motion over long macroevolutionary timescales.

2.3.2 Data

I fitted these two models to empirical data (over 8,000 data points) on phenotypic divergence in various animal lineages over time compiled from studies of contemporary populations and fossils (98 species), and phylogenetic comparative data (583 genera) (Uyeda et al., 2011).

Phenotypic divergence is defined as the darwin numerator, the difference of mean log trait values at different points in time. I filtered out divergence estimates of lineages where evolutionary change was coded as being mediated by human disturbance. I computed mean bootstrapped log-variances of phenotypic divergences for successive time windows with a minimum span of 0.2 Myr to allow extraction of summary statistics. Bootstrapping was performed to give equal weight to all species. Where both fossil and phylogenetic comparative data overlap, I took an average of the two. The phylogenetic comparative data used in this study are not used to estimate the specific number and locations of shifts on a phylogenetic tree, but rather the cumulative effect of these shifts in the form of variation across taxa. Together with the fossil data and data from contemporary populations, they can be used to reconstruct a first-order approximation of the average time course of phenotypic divergence. Uyeda et al. (2011) noted that the phenotypic divergence trajectories indicated by these two datasets run parallel to each other, suggesting that they provide consistent estimates of the rate at which variation in a clade accumulates. While the trajectories run parallel, fossil data indicate more divergence than phylogenetic comparative data at any given point in time, possibly because the selection of fossil lineages studied by palaeontologists is biased towards those that have experienced more evolutionary change. I used a string of mean log-variances of phenotypic divergence for 12 successive time windows as target summary statistics to perform Approximate Bayesian Computation (ABC). This approach allows one to fit models for which the likelihood is intractable and is based on the principle that a set of parameters are more likely if simulating the model with those parameters reproduces closely certain summary statistics computed from the data.

2.3.3 ABC Model Fitting

I used the R package EasyABC v 1.5.2 (Jabot et al., 2023) implementing the method of Lenormand et al. (2013) in the function ABC_sequential. I set nb_simul=100, alpha=0.5 and p_acc_min=0.05. For each model I ran the ABC analysis 10 times independently to obtain combined posterior distributions with 500 samples. For the ECH model I set uniform priors on the Poisson density parameter λ (0-20), the OU drift parameter σ (0-200) and the OU constraint parameter α (0-10^5). For the APS model I set uniform priors on the Poisson density parameter λ (0-20), the OU drift parameter σ (0-200), the OU constraint parameter α (0-10^4) and the expected waiting time between shifts t* (0-10).

To estimate a Bayes factor support for one model over the other from posterior predictive simulations the following approximation was used,

$$BF_{1,2} \approx \frac{P(M1|d(M1,D)<\epsilon)}{P(M2|d(M2,D)<\epsilon)},$$

where d is the Euclidean distance between the simulated summary statistics and the target and ϵ is an arbitrarily small threshold which in this case was set to 1.45.

2.4 Results & Discussion

The joint posterior distributions of parameters of the ECH (Evolutionary Continuum Hypothesis) model and the APS (Adaptive Peak Shift) model are shown in fig. 2.3. The APS model is similar to the phenomenological model used by Uyeda et al. (2011) and indeed posterior parameter estimates are commensurate. Both the ECH and APS model fit the empirical data quite well (fig. 2.3), capturing the relative conservatism of evolution over microevolutionary timescales and the uptick in the accumulation of phenotypic divergence around the 1-million-year mark coinciding with the expected waiting time to macroevolutionary bursts. All the output figures were generated from the posterior distributions of parameter estimates. The ECH model compares favourably to the APS (Adaptive Peak Shift) model as well, despite having one fewer free parameter. The approximate Bayes factor in support of the ECH model versus the APS model is 0.54, suggesting no strong support of one model over the other. These findings suggest that no special processes are necessary to explain burst-like patterns of macro-evolutionary change beyond those that are part of the continuum of processes operating from micro- to macro-evolutionary timescales. This is not to say that adaptive peak shifts may not be important in evolution. The data considered here cannot decide between the two hypotheses, only showing that both are viable explanations for the observed pattern.

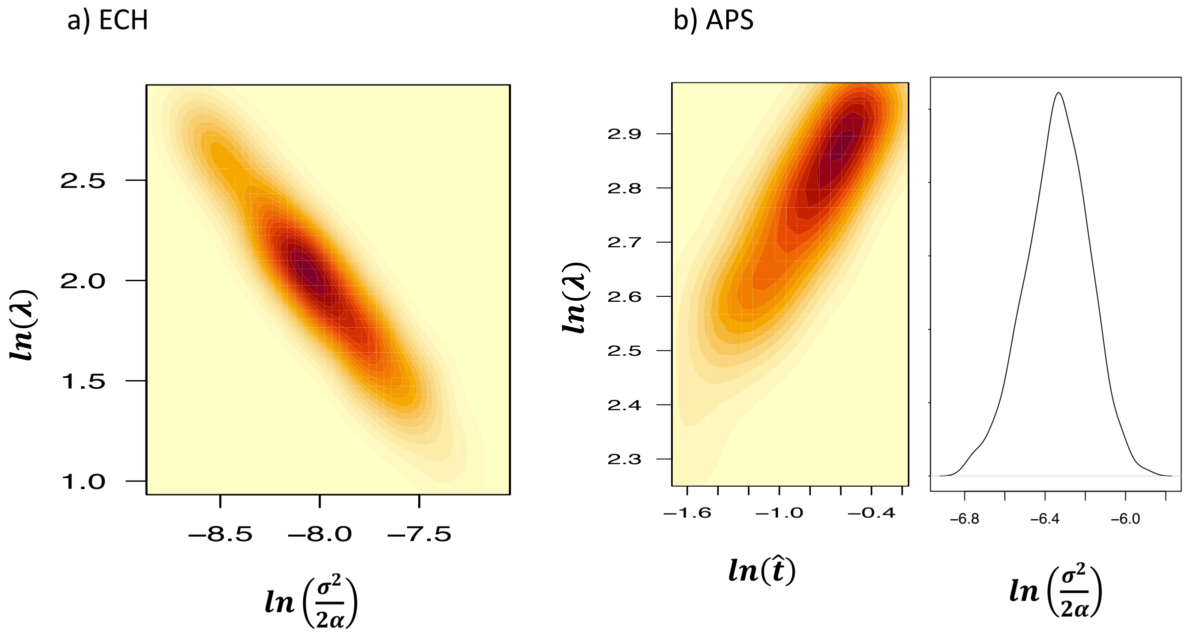

Fig 2.3. Posterior distributions of parameters in the ECH and APS models. On the x axis in the left panel is the natural log of the stationary variance of the OU component. On the y axis is the natural log of the density of adaptive peaks. These components are not entirely identifiable and are related in the posterior by a power law. In the right panel the first subpanel shows the expected waiting time to macroevolutionary bursts on the x-axis and the density of potential shift locations on the y-axis. These components are also related by a power law. In the right subpanel is the posterior density curve for the OU stationary variance within adaptive zones.

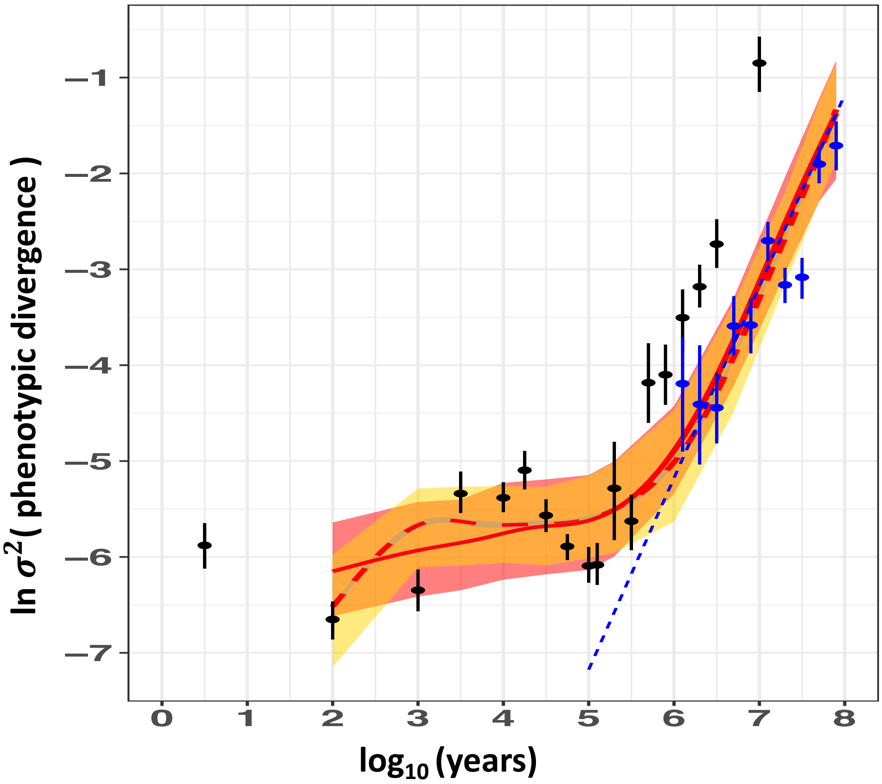

Fig 2.4. Posterior predictive distribution (95% HPD) of the natural log of the variance of phenotypic divergences over time for the ECH model in red and for the APS model in yellow. Points are the observed bootstrapped log variance of phenotypic divergences in successive time windows with associated 95% confidence intervals. Points in black derive from data collected in contemporary animal populations and fossils, while blue points derive from phylogenetic estimates of phenotypic divergence over time. The blue dashed line shows the predicted accumulation of phenotypic divergence over time under a Brownian Motion model applied to phylogenetic comparative data.

The two hypotheses do, however, make some contrasting predictions that could be tested in future. The ECH model predicts that there is a connection between microevolutionary dynamics and the rate of macroevolutionary bursts. Since all evolutionary fluctuations around adaptive peaks are assumed to be part of the same continuum under this model, including fluctuations that lead to crossing the threshold to neighbouring adaptive zones, this predicts that lineages with greater micro-evolutionary fluctuations around adaptive peaks should on average have shorter waiting times to macroevolutionary bursts, hence faster rates of macroevolution. No such relationship is necessarily expected from the APS model since the events triggering an adaptive peak shift are assumed to be disconnected from the typical fluctuations around adaptive peaks. One proxy for the intensity of micro-evolutionary fluctuations might be the amount of intraspecific variation present in morphological traits. Another proxy relevant to the hypothesis that genetic drift plays an important role in these fluctuations is the effective population size.

There are several points not considered in the models explored here. The present models do not consider heterogeneity in evolutionary dynamics across the tree of Life. While the original study from which the empirical data used in this study were sourced found no significant differences in evolutionary dynamics between major clades of vertebrates, this does not exclude the likely possibility that there is significant heterogeneity within these clades. Another source of heterogeneity is the potential heterogeneity in evolutionary dynamics within any lineage over time, such as the fluctuations in population size that likely play a key role in allowing a population to bridge the valleys between adaptive peaks. The key question from the perspective of the ECH is whether population bottlenecks unlocking shifts between adaptive peaks are part of a continuum of fluctuations in population size, or whether they are fundamentally unpredictable from microevolutionary fluctuations across a metapopulation. The present models also do not consider how macroevolutionary bursts are related to speciation. An event that triggers an adaptive peak shift for one population of a species, such as the colonisation of an island or relatively abrupt environmental change in a part of a specie's range, may simultaneously promote speciation if the event also creates barriers to gene flow. Shifts of adaptive peaks or shifts between adaptive peaks in isolated populations can also promote subsequent species persistence by ecological isolation. Indeed, it seems that at least for the evolution of mammal body sizes macroevolutionary bursts of phenotypic evolution are associated with speciation events (Mattila & Bokma, 2008).

These findings shed new light in the debate over punctuated equilibrium (Eldredge & Gould, 1972; Pennell, Harmon & Uyeda, 2014). At least part of the reason why this theory of macroevolution has been and still is so controversial is that it has been assumed that to generate a pattern of punctuational shifts in evolution requires invoking processes taking place over macroevolutionary scales that are separate from the processes shaping evolution over microevolutionary scales. Perhaps this is the case for some lineages. However, relatively abrupt shifts can also emerge in theory as a natural consequence of microevolutionary processes playing out over rugged adaptive landscapes. This study confirms that this process can account for the pattern observed in empirical data, emphasising the importance of the adaptive landscape as a bridge between micro- and macro- evolution (Arnold, Pfrender & Jones, 2001).

2.5 References

1. Arnold, S. J., Pfrender, M. E., & Jones, A. G. (2001). The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica*, *112–113*, 9–32. https://doi.org/10.1023/A:1013373907708
2. Dixit, T., Lund, J., Fulford, A.J.C. *et al.*(2023). Chase-away evolution maintains imperfect mimicry in a brood parasite–host system despite rapid evolution of mimics. *Nature Ecology & Evolution* **7**, 1978–1982. https://doi.org/10.1038/s41559-023-02232-4
3. Eldredge, N., & Jay Gould, S. (1972). Punctuated equilibria: an alternative to phyletic gradualism. In *Models in Palaeobiology* (pp. 82–115).
4. Estes, S., & Arnold, S. J. (2007). Resolving the Paradox of Stasis: Models with Stabilizing Selection Explain Evolutionary Divergence on All Timescales. *The American Naturalist*, *169*(2), 227–244. https://doi.org/10.1086/510633
5. Garcia-Portugues E, Alvarez-Liebana J (2021). Goffda: goodness-of-fit tests for functional data. R v.0.1.0. https://CRAN.R-project.org/package=goffda
6. Hansen, T. F., & Martins, E. P. (1996). Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution*, *50*(4), 1404–1417. https://doi.org/10.1111/j.1558-5646.1996.tb03914.x
7. Jabot F, Faure T, Dumoulin N, Albert C (2023). EasyABC: efficient Approximate Bayesian Computation sampling schemes. R package v.1.5.2. https://CRAN.R-project.org/package=EasyABC
8. Lande, R. (1976). Natural Selection and Random Genetic Drift in Phenotypic Evolution. *Evolution*, *30*(2), 314. https://doi.org/10.2307/2407703
9. Lande, R. (1985). Expected time for random genetic drift of a population between stable phenotypic states. 82. PNAS 82(22), https://doi.org/10.1073/pnas.82.22.7641
10. Landis, M. J., & Schraiber, J. G. (2017). Pulsed evolution shaped modern vertebrate body sizes. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(50), 13224–13229. https://doi.org/10.1073/pnas.1710920114
11. Lenormand, M., Jabot, F., & Deffuant, G. (2013). Adaptive approximate Bayesian computation for complex models. *Computational Statistics*, *28*(6), 2777–2796. https://doi.org/10.1007/s00180-013-0428-3
12. Liem KF (1973). Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. Systematic Zoology. 22:425–441.

13. Lovette, I. J., Bermingham, E., & Ricklefs, R. E. (2002). Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. *Proceedings of the Royal Society B*, *269*(1486), 37-42.
14. Mattila, T. M., & Bokma, F. (2008). Extant mammal body masses suggest punctuated equilibrium. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1648), 2195–2199. https://doi.org/10.1098/rspb.2008.0354
15. Newman C, Cohen J & Kipnis C (1985). Neo-Darwinian evolution implies punctuated equilibria. *Nature*. 400, 400-401
16. Pagel M, O'Donovan C & Meade A (2022). General statistical model shows that macroevolutionary patterns and processes are consistent with Darwinian gradualism. *Nature Communications*. https://doi.org/10.1038/s41467-022-28595-z

17. Pennell, M. W., Harmon, L. J., & Uyeda, J. C. (2014). Is there room for punctuated equilibrium in macroevolution? In *Trends in Ecology and Evolution* (Vol. 29, Issue 1, pp. 23–32). https://doi.org/10.1016/j.tree.2013.07.004
18. Simpson, G. (1944). *Tempo and Mode in Evolution*.
19. Simpson, G. (1953). *The major features of evolution*.
20. Uyeda, J. C., Hansen, T. F., Arnold, S. J., & Pienaar, J. (2011). The million-year wait for macroevolutionary bursts. *Proceedings of the National Academy of Sciences*, *108*(38), 15908–15913. https://doi.org/10.1073/pnas.1014503108
21. Uyeda, J. C., & Harmon, L. J. (2014). A novel Bayesian method for inferring and interpreting the dynamics of adaptive landscapes from phylogenetic comparative data. *Systematic Biology*, *63*(6), 902–918. https://doi.org/10.1093/sysbio/syu057
22. Van Valen L (1973). A new evolutionary law. Evolutionary Theory 1:1-30

Chapter 3

What explains variation in rates of speciation across the tree of Life? A theoretical perspective based on models of protracted speciation.

3.1 Abstract

One might naturally expect a relationship between macroevolutionary rates of speciation and proxies for the rates at which geographical, reproductive and ecological isolation accumulate between incipiently diverging populations. However, recent comparative studies have often failed to find evidence for this prediction. Here I explore a general theoretical explanation for these null results based on analysing the dynamics of protracted speciation models which treat speciation as a process with multiple steps. I find that the expected nonlinearity in the relationship between the macroevolutionary rate of speciation and the rates of component steps in the speciation cycle can lead to only certain steps being strongly rate-limiting while the rates of other steps do not have as much of an impact on the rate of the speciation cycle as a whole. Interaction effects between steps mean that the degree to which a step is rate-limiting depends on the rates of other steps. Nonlinearity and interaction effects predict that the diversification of Life on Earth may often be mathematically chaotic and unpredictable.

3.2 Introduction

There is great variation in rates of speciation across the tree of Life, around the globe and through time (Jetz et al., 2012; Davies et al., 2004; Rabosky et al., 2018). What explains this variation? Many possible predictors have been proposed that vary in their hypothesised connection to different steps of the speciation cycle (Tobias, Ottenburghs & Pigot, 2020). Some degree of geographical isolation is usually thought to be required as an initial step for speciation to get going (Pigot & Tobias, 2015). A subsequent key step in speciation is the evolution of sufficient reproductive isolation to prevent the eventual fusion of two incipient species via hybridisation once they come back into contact (Ritchie, 2007). Finally, for two species to coexist in the long-term theory suggests there must be sufficient ecological isolation between them to prevent eventual extinction of one population through neutral ecological drift or competitive exclusion (Chesson, 2000). One may therefore expect that proxies for the rates of evolution of geographical, reproductive and ecological isolation between incipient species should be correlated with macroevolutionary rates of speciation. However, recent comparative studies have challenged the general truth of this assumption as detailed in the paragraphs below.

The rate of incipient population differentiation, as indicated by population genetic structure, is not correlated with the macroevolutionary rate of speciation across clades of squamate reptiles (Singhal et al., 2022; Singhal et al., 2018) or the rate of diversification in orchids (Kisel et al., 2012), but is correlated with macroevolutionary rates of speciation in New World birds (Harvey et al., 2017). The rate of population differentiation should in theory be related to the rate of geological and climatic changes that produce geographical barriers to gene flow (Hoorn et al., 2010), as well as intrinsic traits of organisms that promote dispersal and colonisation of geographically isolated areas (Smith et al., 2014). Migration and a propensity for dispersal, for instance, have been found to predict higher rates of speciation in birds (Harvey et al., 2017; Phillimore et al., 2006) and higher species richness in fish (Tedesco et al., 2017), while other studies found that an intermediate propensity to disperse promotes diversification (Agnarsson, Cheng & Kuntner, 2014; Paulay & Meyer, 2006). On the other hand, wing morphology indicative of strongly dispersive taxa is not associated with higher rates of diversification in corvoid birds (Kennedy et al., 2016) while being negatively correlated with rates of diversification in the avifauna of the Australasian archipelago (Weeks & Claramunt, 2014) and in the Furnariidae radiation of the Neotropics (Claramunt et al., 2012). Dispersive planktotrophic marine molluscs have lower rates of speciation than their non-planktotrophic and less dispersive counterparts (Jablonski, 1986).

Macroevolutionary speciation rates are decoupled from the rate of evolution of reproductive isolation in *Drosophila* fruit flies and birds (Rabosky and Matute, 2013). Faster evolution of song discrimination in New World passerine birds, a premating reproductive barrier, is also not associated with faster rates of speciation (Freeman et al., 2022). The intensity of sexual selection is thought to be related to the rate of evolution of reproductive isolation, and ultimately to the rate of speciation. However, evidence for a link between proxies of the intensity of sexual selection and rates of speciation remains mixed (Huang & Rabosky, 2014; Cally et al., 2021; Cooney et al., 2017; Janicke et al., 2018). A meta-analysis of earlier work found a small but significant positive association between proxies for the intensity of sexual selection and species richness in clades (Kraaijeveld, Kraaijeveld-Smit & Maan, 2011).

Divergent ecological adaptation may not only promote coexistence between incipient species in sympatry but may in fact promote the evolution of reproductive isolation between populations across disparate taxa (Funk, Nosil & Etges, 2006). Rates of morphological evolution might be taken as a proxy for the rate of divergent ecological adaptation. It does seem that rates of speciation are generally correlated with rates of body size evolution across many clades of vertebrates, but only weakly and inconsistently (Cooney & Thomas, 2021; Rabosky et al., 2013). In a study of divergence in multiple functional traits across more than 1000 allopatric sister vertebrate taxa it was found that divergent ecological adaptation is rare and sister taxa tend in fact to gravitate towards the same adaptive optima (Anderson & Weir, 2022). This contradicts the view that ecological divergence promotes speciation initiation but leaves open the possibility that ecological divergence promotes coexistence later on when sister taxa become sympatric. Another line of evidence for a relationship between the rate of speciation and ecological competition comes from the observation that diversification often slows down as ecological niches are filled (Price et al., 2014; McPeek, 2008; Phillimore & Price, 2008), although there may be other reasons why diversification slows down (Etienne & Rosindell, 2012; Pigot et al., 2010; Condamine, Rolland & Morlon, 2019; Moen & Morlon, 2014). It remains debatable whether speciation generally involves ecological divergence and whether species radiations are always adaptive in nature (Czekanski-Moir & Rundell, 2019).

It is counterintuitive that there are such weak and inconsistent relationships between macroevolutionary rates of speciation and proxies for the rates of evolution of geographical, reproductive and ecological isolation between populations. Rabosky & Matute (2013) and Rabosky (2016) have proposed that a resolution to this apparent paradox is that some steps in the speciation cycle act as 'rate-limiting' steps while the rates of steps that are not rate-limiting are decoupled from the rate of the speciation cycle as a whole. The speciation cycle can be modelled as a protracted process with multiple steps (Rosindell et al., 2010; Etienne & Rosindell, 2012). By mathematically anlysing the dynamics of models of protracted speciation with multiple steps, here I formalise the theoretical argument for why macroevolutionary rates of speciation may be decoupled from the rates of component steps of the speciation cycle.

3.3 Formalising limits on speciation rates with models of protracted speciation

3.3.1 Description of the model

Macroevolutionary models of diversification typically treat speciation as an instantaneous event, yet in reality speciation is a process that takes time to complete. A model that explicitly accounts for this is the 'protracted speciation model' first proposed by Rosindell et al. (2010). I analysed the dynamics of this model and a generalisation with more steps, which I formulate here as class-structured birth-death models for the sake of analytical tractability. In the general model a population can be at one of several sequential stages of the speciation cycle that each have different per capita rates of extinction (μ), rates of transition to subsequent stages (λ) and rates of generation of new populations at earlier stages of the speciation cycle (b). As far as the model is concerned these stages are simply defined in terms of associated parameters, allowing flexibility in the species concept used to define what is and what is not a 'good' species or 'incipient' species.

In the simplest incarnation of the model there are just two stages of the speciation cycle (fig. 1a). A population can be either a good species that fulfils all the criteria of a chosen species concept (G) or an incipient species at an intermediate stage of the speciation cycle (I). The parameter b can be interpreted as the per capita 'rate of speciation initiation' and λ can be interpreted as the per capita 'rate of speciation completion'. I also examined a slightly more complex model where there are three stages of the speciation cycle with two intermediate incipient stages and two associated rates of transition between stages (fig. 1c). I will focus on models with two and three stages to illustrate the general dynamics of this class of models, but the overarching conclusions remain the same for models with any number of intermediate stages in the speciation cycle.

The R code used to generate figures in this paper by simulation from the models can be accessed at https://github.com/LRombaut/protracted_speciation.

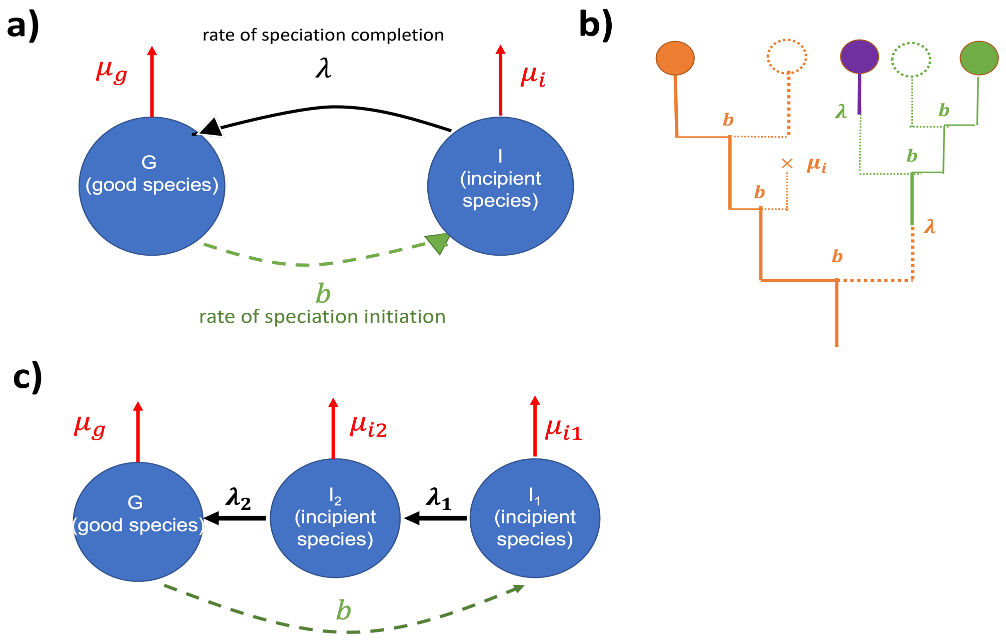

Fig 3.1 Protracted speciation models with two and three steps. The parameter b is the rate of speciation initiation, denoting the formation of new incipient species. The parameters denoted by λ are the rates of transition between stages. The parameters denoted by μ are rates of extinction for incipient and good species. In the phylogenetic diagram on the right solid branches are representative lineages of good species. Each colour denotes a separate species. Dotted branches are lineages of incipient species.

3.3.2 Dynamical Equations

The system of differential equations describing the change in the number of good and incipient species over time is the following for the model with n=2 stages,

$$\begin{aligned} \frac{dG}{dt} &= -\mu_g G + \lambda I \\ \frac{dI}{dt} &= bG - (\mu_i + \lambda) I \end{aligned} \tag{1}$$

For n=3 stages we can derive a similar set of differential equations,

$$\begin{aligned} \frac{dG}{dt} &= -\mu_g G + \lambda I_2 \\ \frac{dI_2}{dt} &= \lambda_1 I_1 - (\mu_{i2} + \lambda_2) I_2 \\ \frac{dI_1}{dt} &= bG - (\mu_{i1} + \lambda_1) I_1 \end{aligned} \tag{2}$$

For n stages the general system of differential equations can be expressed as follows,

$$\frac{d\vec{n}}{dt} = \begin{pmatrix} \frac{dG}{dt} \\ \frac{dI_j}{dt} \\ \vdots \\ \frac{dI_2}{dt} \\ \frac{dI_1}{dt} \end{pmatrix} = \begin{pmatrix} -\mu_G & \lambda_j I_j & \cdots & 0 & 0 \\ 0 & -(\mu_{ij} + \lambda_j) & & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & & -(\mu_{i2} + \lambda_2) & \lambda_1 I_1 \\ b_G & b_{ij} & \cdots & b_{i2} & -(\mu_{i1} + \lambda_1) \end{pmatrix} \begin{pmatrix} G \\ I_j \\ \vdots \\ I_2 \\ I_1 \end{pmatrix}$$

$$\frac{d\vec{n}}{dt} = \boldsymbol{R}\vec{n} \tag{3}$$

The vector $\vec{n}$ contains the numbers of good and incipient species in a clade and the matrix $\boldsymbol{R}$ contains the birth, death and transition rate parameters. This is a model of class-structured growth where there is a constant probability of transition between classes that is independent of time and a population's history.

The general solution for an initial number of good and incipient species in a clade $\vec{n}_0$ is then,

$$\vec{n}(t) = e^{\boldsymbol{R}t}\vec{n}_0 \qquad (4)$$

The solution can be expressed in terms of the eigenvectors ($\vec{v}$) and associated eigenvalues (δ) of the matrix **R.** For n=2 stages there is the following solution as an example,

$$\vec{n}(t) = \begin{pmatrix} G(t) \\ I(t) \end{pmatrix} = c_1 e^{\delta^1 t}\,\overrightarrow{v_1} + \; c_2 e^{\delta^2 t}\,\overrightarrow{v_2} \qquad (5)$$

Here, c_1 and c_2 are weights determined by the initial number of good and incipient species. The largest eigenvalue of the parameter matrix **R** is denoted by δ^1, and $\overrightarrow{v_1}$ is its associated eigenvector. The contributions of the remaining eigenvectors relative to the leading eigenvector decay exponentially with time, so in the long term the per capita rate of growth of a clade is dominated by the leading eigenvalue of the parameter matrix **R**. For the model with n=2 stages in the speciation cycle we can apply the quadratic formula to the characteristic polynomial of the matrix **R** to explicitly solve for the leading eigenvalue,

$$\boldsymbol{\delta^1} = \frac{1}{2}\left(-(\mu_g + \mu_i + \lambda) + \sqrt{(\mu_g + \mu_i + \lambda)^2 - 4\left(\mu_g\mu_i - \lambda(b - \mu_g)\right)}\right) \qquad (6)$$

For a model with n stages generally, the leading eigenvalue will be a nonlinear function of the parameters of the model.

3.4 Biological implications of the protracted speciation model

3.4.1 Inertial diversification and the ratio of good species to incipient species in a clade

At least in the short term the trailing eigenvectors can contribute significantly to the diversification dynamics predicted by the model if the ratio of good species to incipient species is out of equilibrium (fig. 2a). The dominant or leading eigenvalue is the largest numerical eigenvalue while all other eigenvalues are the trailing ones. In reality this situation could arise if the parameters that describe the speciation cycle change suddenly due to a change in the environment, or if a mass extinction event disproportionately affects the number of populations at certain stages of the speciation cycle. The trailing eigenvectors introduce some inertia to changes in the dynamics of the speciation cycle. Populations at intermediate stages of the speciation cycle act like a reservoir of diversity, taking time both to fill as well as empty to a new equilibrium level. Ultimately however, the ratio of good species to incipient species will approach an equilibrium at which the number of good and incipient species increases or decreases at a fixed ratio given by the leading eigenvector of the model parameter matrix (eqn. 3) (fig. 2b). For the model with n=2 stages this ratio of good species to incipient species is,

$$1 : \frac{b-\mu_g-\delta^1}{\mu_i+\delta^1} \qquad (7)$$

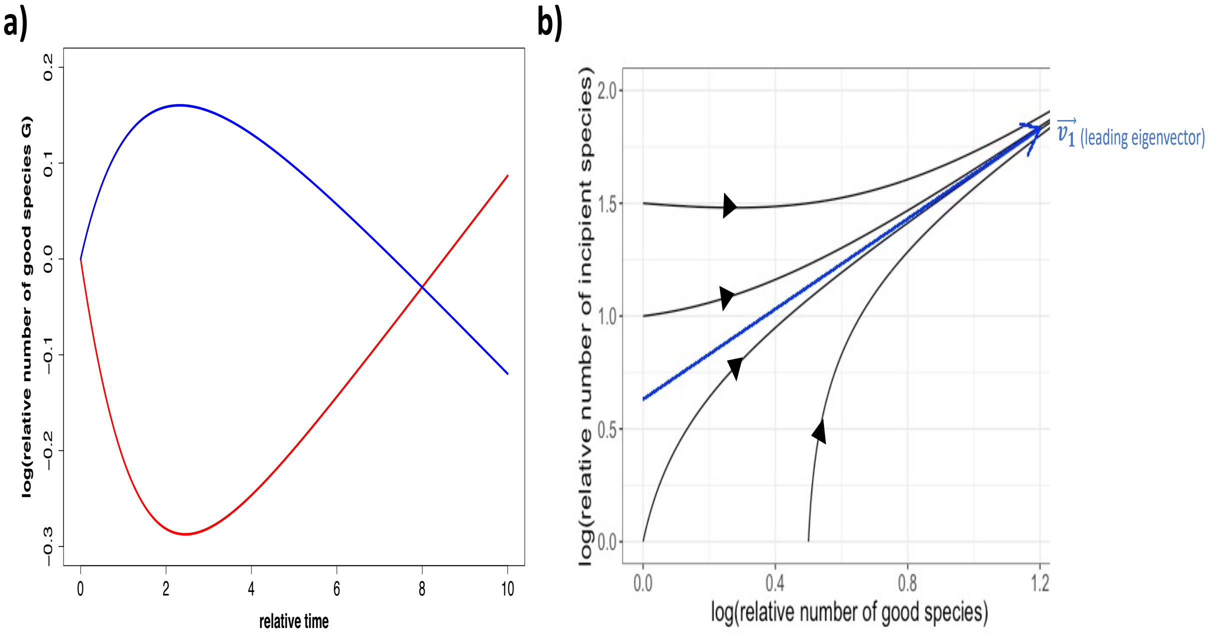

Fig 3.2. (a) Transient dynamics of speciation when the ratio of good species to incipient species is out of equilibrium. In the blue scenario there are initially many incipient species for every good species, so the number of good species continues to accumulate at first, but eventually the number of incipient species that can produce good species declines. In the red scenario there are initially few incipient species for every good species, so the number of good species declines at first, but eventually the number of incipient species that can produce good species increases. In both cases, the rate of change in the number of good species eventually settles down to a steady increase or decrease over time as shown in (b).

3.4.2 Nonlinearity, interaction effects and chaotic diversification dynamics

The relationship between parameters in the speciation cycle and the expected macroevolutionary speciation rate is nonlinear, encompassing interaction effects between parameters (fig. 3.3). By interaction effects is meant that the effect of changing a parameter depends on the state of other parameters. We can infer from these observations that nonlinearity and interaction effects should be expected by default in the relationship between the rate of speciation and any proxy predictors for the rates of different steps in the speciation cycle such as those mentioned in the introduction. Consequently, for at least some parts of the model parameter space there will be certain predictors of speciation rate that appear essentially uncorrelated with the macroevolutionary rate of speciation because they are not associated with the most strongly rate-limiting steps in the speciation cycle.

For a given range of parameter space the macroevolutionary rate of speciation may be fairly robust to perturbations in parameters that are not the rate-limiting parameters, while for other parts of parameter space the macroevolutionary rate of speciation may be quite sensitive to the same perturbations in those parameters (fig. 3.4). Chaotic and unpredictable diversification dynamics may therefore be expected. This may go some way towards accounting for the apparently unpredictable waxing and waning of some clades as they are buffeted by changes through geological time.

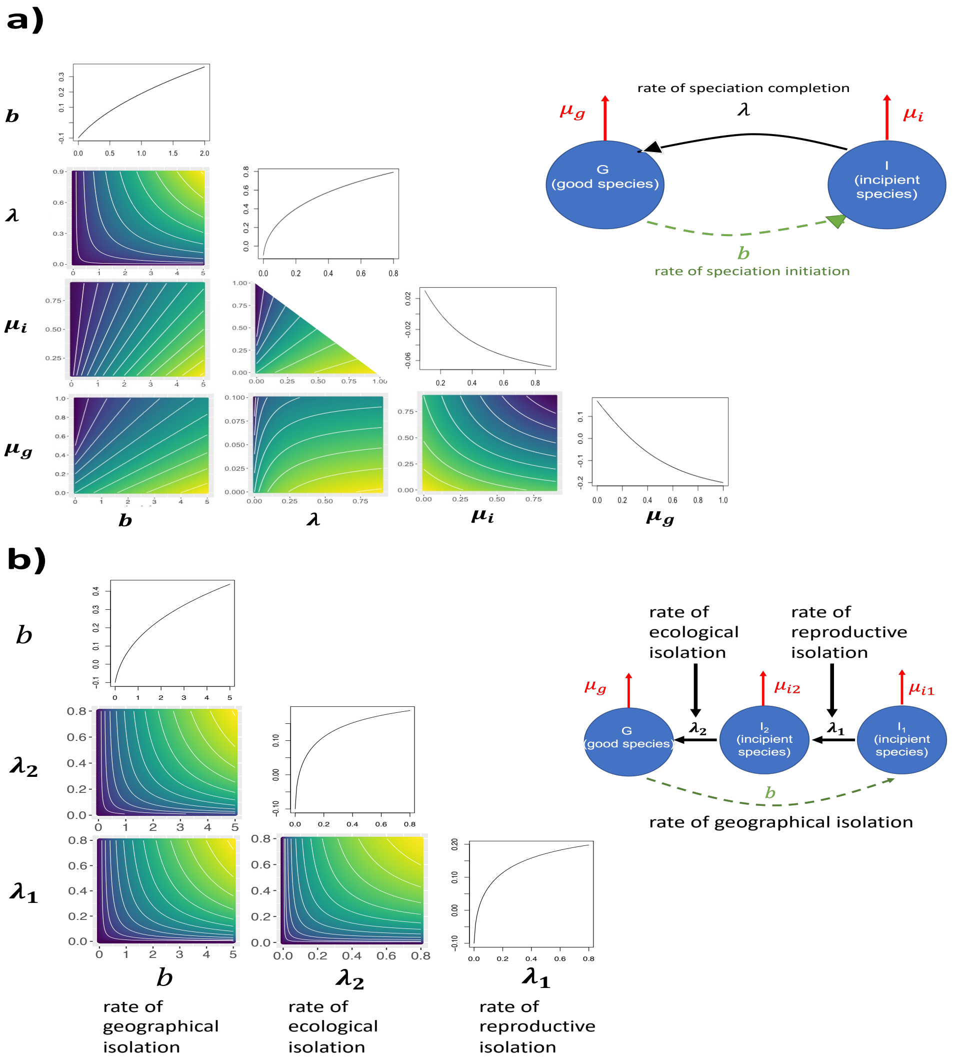

Fig 3.3. Relationship between parameters of the models and the expected rate of diversification. The diagonal plots show the expected relationship with the rate of diversification on the y axis when a single parameter is varied at a time, keeping all other parameters constant. Contour plots show the interactions between two parameters at a time, with lighter colours indicating a higher expected rate of diversification. The rates of geographical, reproductive and ecological steps are connected to different steps in panel b as an example.

3.4.3 Caveats of the model

An important caveat in testing predictions of the model is that the global nonlinearity and interaction effects predicted may not be apparent in empirical data if the range of observed variation in speciation rates is highly restricted. For any small neighbourhood of parameter space, the relationship between macroevolutionary rates of speciation and predictors of those rates may appear essentially flat or linear. There is, however, already an empirical hint of this nonlinearity to be found in supplementary figure 3 in Etienne, Morlon & Lambert (2014) who contrasted the macroevolutionary rate of speciation estimated from phylogenies across 80 bird clades with the speciation completion rates estimated from the same phylogenies. Another caveat of the modelling framework used here is that it assumes that when any good species becomes extinct, any orphaned incipient species that descend from that good species remain incipient species in terms of their liability to go extinct and transition to becoming good species. One may rather assume that a representative lineage of this group of incipient species becomes a fully fledged good species. A final caveat to note is that I have not considered the possibility that incipient species themselves are able to produce new incipient species, adding extra terms to the dynamical equations analysed in this study. This would decouple the dynamics of part of the speciation cycle, reducing the effect of rate-limitation. This scenario may pertain to exceptionally rapid radiations producing species flocks such as those of cichlid fishes in the African Great Lakes.

3.4.4 Prospects for comparative speciation research

The theoretical insights presented in this paper suggest some future lines of inquiry for the field of comparative speciation research. It would be informative to empirically gain insight into variation in the rates of different components of the speciation cycle, not just the macroevolutionary rate of the speciation cycle as a whole. The protracted speciation model with two states can already be fit to empirical phylogenies, although some parameters cannot be estimated without bias, may be unidentifiable, or may be estimated inaccurately in small trees (Lambert, Morlon & Etienne, 2015; Simonet et al., 2018; Etienne, Morlon & Lambert, 2014). Recently, a method has been proposed that can more reliably estimate many parameters of the protracted speciation model based on lineage-level phylogenies with resolution below the species level (Hua, Herdha & Burden, 2022). The findings of this paper predict that there should be a nonlinear relationship between predictors of speciation rate and any proxy predictors of such rates. Given the predictions highlighted in this paper, a point that deserves more attention is testing for nonlinearity and interaction effects in multi-predictor models of macroevolutionary speciation rates. Ultimately, the goal of comparative speciation research is to better understand which intrinsic traits of organisms and extrinsic features of the environment explain variation in macroevolutionary rates of speciation, as well as the rates of different steps of the speciation cycle. It is quite possible that the most strongly rate-limiting steps may turn out to differ between clades and taxonomic scales.

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3.6 References

1. Agnarsson, I., Cheng, R. C., & Kuntner, M. (2014). A multi-clade test supports the intermediate dispersal model of biogeography. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086780
2. Anderson, S. A. S., & Weir, J. T. (2022). The role of divergent ecological adaptation during allopatric speciation in vertebrates. *Science*, *378*, 1214–1218.

3. Cally, J. G., Stuart-Fox, D., Holman, L., Dale, J., & Medina, I. (2021). Male-biased sexual selection, but not sexual dichromatism, predicts speciation in birds. *Evolution*, *75*(4), 931–944. https://doi.org/10.1111/evo.14183
4. Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, *31*, 343–366.
5. Claramunt, S., Derryberry, E. P., Remsen, J. v., & Brumfield, R. T. (2012). High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1733), 1567–1574. https://doi.org/10.1098/rspb.2011.1922
6. Condamine, F. L., Rolland, J., & Morlon, H. (2019). Assessing the causes of diversification slowdowns: temperature-dependent and diversity-dependent models receive equivalent support. *Ecology Letters*, *22*(11), 1900–1912. https://doi.org/10.1111/ele.13382
7. Cooney, C. R., & Thomas, G. H. (2021). Heterogeneous relationships between rates of speciation and body size evolution across vertebrate clades. *Nature Ecology and Evolution*, *5*(1), 101–110. https://doi.org/10.1038/s41559-020-01321-y
8. Cooney, C. R., Tobias, J. A., Weir, J. T., Botero, C. A., & Seddon, N. (2017). Sexual selection, speciation and constraints on geographical range overlap in birds. *Ecology Letters*, *20*(7), 863–871. https://doi.org/10.1111/ele.12780
9. Czekanski-Moir, J. E., & Rundell, R. J. (2019). The ecology of nonecological speciation and nonadaptive radiations. *Trends in Ecology and Evolution*, *34*(5), 400–415. https://doi.org/10.1016/j.tree.2019.01.012
10. Davies, T. J., Barraclough, T. G., Chase, M. W., Soltis, P. S., Soltis, D. E., & Savolainen, V. (2004). Darwin's abominable mystery: Insights from a supertree of the angiosperms. *PNAS*, *101*(7), 1904–1909.
11. Etienne, R. S., Morlon, H., & Lambert, A. (2014). Estimating the duration of speciation from phylogenies. *Evolution*, *68*(8), 2430–2440. https://doi.org/10.1111/evo.1243
12. Etienne, R. S., & Rosindell, J. (2012). Prolonging the past counteracts the pull of the present: protracted speciation can explain observed slowdowns in diversification. *Systematic Biology*, *61*(2), 204–213. https://doi.org/10.1093/sysbio/syr091
13. Freeman, B. G., Rolland, J., Montgomery, G. A., & Schluter, D. (2022). Faster evolution of a premating reproductive barrier is not associated with faster speciation rates in New World passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1966), 20211514. https://doi.org/10.1098/rspb.2021.1514
14. Funk, D. J., Nosil, P., & Etges, W. J. (2006). Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *PNAS* , *103*(9), 3209–3213.
15. Harvey, M. G., Seeholzer, G. F., Smith, B. T., Rabosky, D. L., Cuervo, A. M., & Brumfield, R. T. (2017). Positive association between population genetic differentiation and speciation rates in New World birds. *PNAS*, *114*(24), 6328–6333. https://doi.org/10.1073/pnas.1617397114
16. Hoorn, C., Wesselingh, F. P., ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C. L., Figueiredo, J. P., Jaramillo, C., Riff, D., Negri, F. R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T., & Antonelli, A. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, *330*, 927–931. https://www.science.org/doi/10.1126/science.1194585

17. Hua, X., Herdha, T., & Burden, C. (2022). Protracted speciation under the state-dependent speciation and extinction approach. *Systematic Biology*. https://doi.org/10.1093/sysbio/syac041
18. Huang, H., & Rabosky, D. L. (2014). Sexual selection and diversification: reexamining the correlation between dichromatism and speciation rate in birds. *The American Naturalist*, *184*(5), E101–E114. https://doi.org/10.1086/678054
19. Jablonski, D. (1986). Larval ecology and macroevolution in marine invertebrates. *Bulletin of Marine Science*, *39*(2), 565–587.
20. Jablonski, D. (2008). Species selection: theory and data. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 501–524. https://doi.org/10.1146/annurev.ecolsys.39.110707.173510
21. Janicke, T., Ritchie, M. G., Morrow, E. H., & Marie-Orleach, L. (2018). Sexual selection predicts species richness across the animal kingdom. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1878). https://doi.org/10.1098/rspb.2018.0173
22. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature*, *491*(7424), 444–448. https://doi.org/10.1038/nature11631
23. Kennedy, J. D., Borregaard, M. K., Jønsson, K. A., Marki, P. Z., Fjeldså, J., & Rahbek, C. (2016). The influence of wing morphology upon the dispersal, geographical distributions and diversification of the corvides (Aves; Passeriformes). *Proceedings of the Royal Society B: Biological Sciences*, *283*(1844). https://doi.org/10.1098/rspb.2016.1922
24. Kisel, Y., Moreno-Letelier, A. C., Bogarín, D., Powell, M. P., Chase, M. W., & Barraclough, T. G. (2012). Testing the link between population genetic differentiation and clade diversification in Costa Rican orchids. *Evolution*, *66*(10), 3035–3052. https://doi.org/10.1111/j.1558-5646.2012.01663.x
25. Kraaijeveld, K., Kraaijeveld-Smit, F. J. L., & Maan, M. E. (2011). Sexual selection and speciation: the comparative evidence revisited. *Biological Reviews*, *86*(2), 367–377. https://doi.org/10.1111/j.1469-185X.2010.00150.x
26. Lambert, A., Morlon, H., & Etienne, R. S. (2015). The reconstructed tree in the lineage-based model of protracted speciation. *Journal of Mathematical Biology*, *70*(1–2), 367–397. https://doi.org/10.1007/s00285-014-0767-x
27. McPeek, M. A. (2008). The ecological dynamics of clade diversification and community assembly. *American Naturalist*, *172*(6). https://doi.org/10.1086/593137
28. Moen, D., & Morlon, H. (2014). Why does diversification slow down? *Trends in Ecology and Evolution*, *29*(4), 190–197. https://doi.org/10.1016/j.tree.2014.01.010
29. Paulay, G., & Meyer, C. (2006). Dispersal and divergence across the greatest ocean region: do larvae matter? *Integrative and Comparative Biology*, *46*(3), 269–281. https://doi.org/10.1093/icb/icj027
30. Phillimore, A. B., Freckleton, R. P., David, C., Orme, L., & Owens, I. P. F. (2006). Ecology predicts large-scale patterns of phylogenetic diversification in birds. *American Naturalist*, *168*(2), 220–229.
31. Phillimore, A. B., & Price, T. D. (2008). Density-dependent cladogenesis in birds. *PLoS Biology*, *6*(3), 0483–0489. https://doi.org/10.1371/journal.pbio.0060071
32. Pigot, A. L., Phillimore, A. B., Owens, I. P. F., & Orme, C. D. L. (2010). The shape and temporal dynamics of phylogenetic trees arising from geographic speciation. *Systematic Biology*, *59*(6), 660–673. https://doi.org/10.1093/sysbio/syq058
33. Pigot, A. L., & Tobias, J. A. (2015). Dispersal and the transition to sympatry in vertebrates. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1799). https://doi.org/10.1098/rspb.2014.1929

34. Price, T. D., Hooper, D. M., Buchanan, C. D., Johansson, U. S., Tietze, D. T., Alström, P., Olsson, U., Ghosh-Harihar, M., Ishtiaq, F., Gupta, S. K., Martens, J., Harr, B., Singh, P., & Mohan, D. (2014). Niche filling slows the diversification of Himalayan songbirds. *Nature*, *509*(7499), 222–225. https://doi.org/10.1038/nature13272
35. Price-Waldman, R. M., Shultz, A. J., & Burns, K. J. (2020). Speciation rates are correlated with changes in plumage color complexity in the largest family of songbirds. *Evolution*, *74*(6), 1155–1169. https://doi.org/10.1111/evo.13982
36. Rabosky, D. L. (2016). Reproductive isolation and the causes of speciation rate variation in nature. *Biological Journal of the Linnean Society*, *118*(1), 13–25. https://doi.org/10.1111/bij.12703
37. Rabosky, D. L., Chang, J., Title, P. O., Cowman, P. F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T. J., Coll, M., & Alfaro, M. E. (2018). An inverse latitudinal gradient in speciation rate for marine fishes. *Nature*, *559*(7714), 392–395. https://doi.org/10.1038/s41586-018-0273-1
38. Rabosky, D. L., & Matute, D. R. (2013). Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in Drosophila and birds. *PNAS*, *110*(38), 15354–15359. https://doi.org/10.1073/pnas.1305529110
39. Rabosky, D. L., Santini, F., Eastman, J., Smith, S. A., Sidlauskas, B., Chang, J., & Alfaro, M. E. (2013). Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications*, *4*, 1–8. https://doi.org/10.1038/ncomms2958
40. Ritchie, M. G. (2007). Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 79–102. https://doi.org/10.1146/annurev.ecolsys.38.091206.095733
41. Rosindell, J., Cornell, S. J., Hubbell, S. P., & Etienne, R. S. (2010). Protracted speciation revitalizes the neutral theory of biodiversity. *Ecology Letters*, *13*(6), 716–727. https://doi.org/10.1111/j.1461-0248.2010.01463.x
42. Simonet, C., Scherrer, R., Rego-Costa, A., & Etienne, R. S. (2018). Robustness of the approximate likelihood of the protracted speciation model. *Journal of Evolutionary Biology*, *31*(3), 469–479. https://doi.org/10.1111/jeb.13233
43. Singhal, S., Huang, H., Grundler, M. R., Marchán-Rivadeneira, M. R., Holmes, I., Title, P. O., Donnellan, S. C., & Rabosky, D. L. (2018). Does population structure predict the rate of speciation? A comparative test across Australia's most diverse vertebrate radiation. *American Naturalist*, *192*(4), 432–447. https://doi.org/10.1086/699515
44. Singhal, S., Colli, G. R., Grundler, M. R., Costa, G. C., Prates, I., & Rabosky, D. L. (2022). No link between population isolation and speciation rate in squamate reptiles. *PNAS*, *119*(4). https://doi.org/10.1073/pnas.2113388119
45. Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C. D., Pérez-Emán, J., Burney, C. W., Xie, X., Harvey, M. G., Faircloth, B. C., Glenn, T. C., Derryberry, E. P., Prejean, J., Fields, S., & Brumfield, R. T. (2014). The drivers of tropical speciation. *Nature*, *515*(7527), 406–409. https://doi.org/10.1038/nature13687
46. Tedesco, P. A., Paradis, E., Lévêque, C., & Hugueny, B. (2017). Explaining global-scale diversification patterns in actinopterygian fishes. *Journal of Biogeography*, *44*(4), 773–783. https://doi.org/10.1111/jbi.12905
47. Tobias, J. A., Ottenburghs, J., & Pigot, A. L. (2020). Avian Diversity: Speciation, Macroevolution, and Ecological Function. *The Annual Review of Ecology, Evolution and Systematics*, *51*, 533–560. https://doi.org/10.1146/annurev-ecolsys-110218

48. Weeks, B. C., & Claramunt, S. (2014). Dispersal has inhibited avian diversification in Australasian archipelagoes. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1791), 2–8. https://doi.org/10.1098/rspb.2014.1257

Chapter 4

Allometric Conservatism in the Evolution of Bird Beaks.

4.1 Abstract

Evolution can involve periods of rapid divergent adaptation and expansion in the range of diversity, but evolution can also be relatively conservative over certain timescales due to functional, genetic-developmental, and ecological constraints. One way in which evolution may be conservative is in terms of allometry, the scaling relationship between the traits of organisms and body size. Here, we investigate patterns of allometric conservatism in the evolution of bird beaks with beak size and body size data for a representative sample of over 5000 extant bird species within a phylogenetic framework. We identify clades in which the allometric relationship between beak size and body size has remained relatively conserved across species over millions to tens of millions of years. We find that allometric conservatism is nonetheless punctuated by occasional shifts in the slopes and intercepts of allometric relationships. A steady accumulation of such shifts through time has given rise to the tremendous diversity of beak size relative to body size across birds today. Our findings are consistent with the Simpsonian vision of macroevolution, with evolutionary conservatism being the rule but with occasional shifts to new adaptive zones.

4.2 Introduction

George Gaylord Simpson was an influential contributor to the theory of macroevolution (Simpson 1944, 1953). Simpson proposed that the majority of evolution takes place within "adaptive zones," bounded regions of trait space characterized by relatively constrained rates of directional trait evolution, in which species evolve to partition sets of available niches. According to Simpson, this pattern of evolution is punctuated by occasional shifts to new adaptive zones triggered by ecological opportunity in new environments or following mass extinctions, or they may be spurred by the evolution of key innovations in functional traits. Taken together, this implies an overall pattern of generally conservative evolution, broken by occasional bursts of rapid directional evolution over long macroevolutionary or "megaevolutionary" timescales.

One way in which evolution may be conservative is in terms of allometry, a scaling relationship between the traits of organisms and body size following a power law of the form $y=Axb$ (Huxley 1932; Lande 1979). Allometry is a ubiquitous feature of biological variation, both within species and across species from micro- to macroevolutionary levels (Conner et al. 2014; Voje et al. 2014; Outomuro and Johansson 2017). Trait allometry across species emerges from constraints on evolution. These can be classified into genetic-developmental, functional, and ecological constraints. When genetic-developmental constraints are conserved across species, evolutionary allometry may emerge from allometry in the growth and development of the organism (Huxley 1932). Stabilizing selection on the function of traits can also help to maintain allometric conservatism in trait evolution (e.g., Skandalis et al. 2017). Ultimately, limits on ecological opportunity may prevent lineages from breaking free from conserved allometric relationships to adapt to new niches (e.g., Grant and Grant 2006).

As a consequence of genetic-developmental, functional, and ecological constraints, allometric relationships may be conserved over timescales spanning millions to tens of millions of years (Voje et al. 2014; Houle et al. 2019). At one extreme is the possibility that lineages within major taxa evolve according to a strict Simpsonian model of evolution in which the allometric relationships between traits across different clades are strongly conserved through time and species in different clades rarely converge on the same trait combinations. In this scenario, variation between clades outweighs variation within clades. At the other extreme is the possibility that shifts in allometric relationships are so frequent that all lineages effectively evolve under the same loose allometric regime with extensive convergent evolution. Under this evolutionary scenario, variation within major clades can outweigh variation between clades. Observed evolutionary patterns may lie somewhere between these two qualitative extremes. At the scale of an entire class of organisms, measuring the extent and phylogenetic distribution of allometric conservatism is key to understanding the macroevolutionary dynamics that give rise to present day diversity.

The bird beak is an interesting anatomical trait on which to study evolutionary allometric conservatism. Bird beaks span a great range of sizes relative to body size, from the relatively long beaks of small hummingbirds to the relatively short beaks of ostriches. This diversity is closely associated with adaptation to different ecological niches (Pigot et al. 2020). In birds, there is strong allometry within certain clades between the shape and size of the beak (Bright et al. 2016, 2019; Navalón et al. 2020), and also between the size of the beak and body size (Van Den Elzen and Nemeschkal 2007; Shatkovska and Ghazali 2020). This pattern is scale dependent, however. Correlation between beak shape and size is relatively much weaker across major bird clades than it is within bird clades (Felice and Goswami 2018). This implies a breakdown in allometric conservatism on megaevolutionary timescales through multiple shifts in allometric relationships.

It remains unclear where on the bird phylogeny shifts in allometric relationships have taken place and what is the pattern of shifts through time that has ultimately produced the striking diversity of beak size in relation to body size we observe today across the world's birds. In this study, we aim to localize shifts in the allometric relationship between beak size and body size across the branches of the bird phylogeny. Using these inferred shifts, we test whether disparity in allometric relationships among bird clades expanded early in the history of crown birds with a subsequent deceleration, consistent with expectations under an adaptive radiation model in the wake of the ecological vacuum left by the K-Pg mass extinction.

4.3 Methods

4.3.1 Morphological Data

We extracted bill centroid size measurements (mm) from landmarked three-dimensional scans of the bills of museum specimens. These scans were previously obtained as part of a broader project and details of specimen selection, scanning, and landmarking can be found in Cooney et al. (2017). Typically, only one adult male individual was sampled per species. Centroid size is defined as the square root of the sum of squared Euclidean distances between each landmark and their centroid. Bill length, width, and depth were also measured for a majority of the same specimens and we found a very close relationship between these linear dimensions and bill centroid size in a multiple linear regression with the log-linear dimensions being the predictors and log bill centroid size being the response ($R^2 = 0.97$).

We obtained body mass data (g) for males of each species from the species-level medians reported by Myhrvold et al. (2015), as well as raw body mass data from museum records (www.vertnet.org). For the latter, we performed an automated error screening and removed 453 out of 28,355 anomalous records that were likely the result of human error. We then calculated species-level averages by taking the mean of log-transformed data. We computed a mean of the two sources for each species, weighted by sample size. The median total sample size per species was three individuals.

4.3.2 Phylogeny

We downloaded a sample of 1000 “Hackett stage 2” trees from www.birdtree.org (Jetz et al. 2012), representing the posterior distribution of phylogenetic relationships among 9993 extant bird species based on molecular sequence data plus taxonomic imputation. We constructed a maximum clade credibility (MCC) tree from this sample of trees using TreeAnnotator (Bouckaert et al. 2019). Clades of the MCC tree were grafted onto corresponding nodes of the Prum et al. (2015) dated backbone phylogeny, as described in Cooney et al. (2017). The Prum et al. (2015) backbone phylogeny has posterior probability support for all but one of the nodes equal to 1.0. We mapped taxonomic labels for all other data to the species names in Jetz et al. (2012) with the aid of synonym tables from Avibase (www.avibase.org).

4.3.3 Shifts in Allometric Relationships

To infer shifts in allometric relationships across the phylogeny, we used the rjMCMC method implemented in bayou (Uyeda and Harmon 2014; Uyeda et al. 2017). Bayou can fit models with multiple evolutionary regimes, each following an Ornstein-Uhlenbeck (OU) process of trait evolution with a primary optimum parameterized by an allometric intercept θ and slope β. The relationship between bill centroid size (lnC) and body size (lnM) of species j is modeled as $\ln C_j = \mathbf{w}_{j,a} \ \boldsymbol{\theta} + \beta_j \ln M_j$, where $\mathbf{w}_{j,a}$ is a row vector of weights for each regime and $\boldsymbol{\theta}$ is a column vector of all intercepts in the history of allometric regimes in which the lineage has evolved. Although shifts in allometry are modeled as discrete events, traits are assumed to evolve toward their new optima gradually at a rate proportional to a. The OU process is parameterized in terms of a, the evolutionary constraint parameter, and σ^2, the evolutionary rate parameter, but for each major clade in Table S1 we instead report the phylogenetic half-life ($\ln(2)\boldsymbol{a}$), a measure of phylogenetic signal for traits evolving under an OU process, and the estimated stationary variance of the OU process ($\boldsymbol{\sigma}22\boldsymbol{a}$), a measure of the expected residual variance around the allometric axis within regimes. These derived metrics are more readily interpretable. For further elaboration on the model and software implementation, see Uyeda et al. (2017).

We note that a Brownian motion process (BM) is a special case of an OU process with $a = 0$. We therefore did not fit a separate multi-regime BM model to our data. Although intraspecific error can erode phylogenetic signal in data generated under a BM process and give the appearance that species have evolved under an OU process (Cooper et al. 2016), we find that our estimates of phylogenetic half-life are robust to reasonable assumptions about intraspecific error (see below).

Because an analysis on our full dataset of 5083 species proved to be computationally intractable in any reasonable time frame, we split our data into 18 clades of up to 800 species (see Table S1) and ran analyses on these clades in parallel. A limitation of this approach is that we cannot explicitly test for shifts at the base of these clades. Instead, we compare the 95%HPD for the parameters at the root of each clade to the parameters inferred under a global model that reflects an average allometric relationship (see below).

For each clade-level analysis, we set a Poisson prior distribution on the number of allometric shifts with a λ parameter of 2% of the number of species in that clade rounded up to the nearest integer. We placed a uniform prior on the probability of a shift over all branches on the phylogeny and also on the locations of shifts along branches. We set half-Cauchy prior distributions on a and σ^2 with a scale parameter of 0.1. We set weakly informative normal priors on the slopes (mean = 0.33, SD = 0.5) and intercepts (mean = 2, SD = 0.5) of each regime. The slope prior is based on an isometric relationship between bill size and body mass. Because bill centroid size has dimensions of length while body mass may be assumed to be proportional to volume that has dimensions of length cubed, an allometric slope of ~1/3 is consistent with isometry, meaning that relative bill size stays the same as body size increases across species. A slope greater than 1/3 implies positive allometry, meaning that bills become relatively larger as body size increases across species. A slope less than 1/3 implies negative allometry, meaning that bills become relatively smaller as body size increases across species. If slope is held constant, the allometric intercept reflects the relative size of the bill for a given body size.

For each clade-level analysis, we ran replicate MCMC chains in parallel, sampling every 200 generations until convergence was reached with a burnin proportion of 50%. For Coraciimorphae and Aequorlitornithes, we ran four replicate chains for 100 million generations each. We ran four replicate chains for 60 million generations each for Accipitriformes, Columbaves, Galliformes, Nectariniidae, Passeroidea, and Sylvioidea. For the remaining clades, we ran duplicate chains for 30 million generations each. We also fit a model with a single allometric regime across all birds. Duplicate chains for this model were run for 2 million generations with a burnin proportion of 20%.

Parameters monitored for convergence include the likelihood, the number of inferred shifts and their locations, as well as a and σ^2. To conclude that the chains had reached convergence, we required an effective sample size greater than 200 for each parameter in each chain and a value of Gelman and Rubin's R diagnostic below 1.05 (Gelman and Rubin 1992). For some clades, the traces of the R statistic for a and σ^2 followed a somewhat erratic pattern. We speculate that this may be due to the identifiability issue inherent in jointly estimating a and σ^2 and/or a consequence of the nature of the half-Cauchy prior distributions on these parameters generating large outliers. In any case, we found that the posterior distributions for a and σ^2 were identical between chains in the bulk of the distribution and only differed in terms of outliers in the tails.

To identify the locations of shifts, we visualized the distribution of posterior probability support for the presence of shifts across the branches of the phylogeny. Because support for a shift may sometimes be “smeared” across a set of adjacent branches, rather than relying on a posterior probability cutoff per branch to infer shifts we first identified regions with elevated support for the presence of a shift and then we located shifts on branches with the maximum posterior probability for a shift. Where the posterior probability for a shift was similar between adjacent branches, preference was given to the most rootward branch encompassing the possibility that the shift occurred along a descendent branch. We used the posterior distribution on the number of shifts as a guide to how many shifts there were to identify in each clade. Using these fixed shift locations, we estimated the allometric slope and intercept across evolutionary regimes by rerunning bayou chains in duplicate for 20 million generations for each of the 18 major clades. We assessed convergence as per the first set of analyses.

4.3.4 Measurement Error and Sensitivity Analyses

We performed sensitivity analyses to gauge the impact of intraspecific variation in bill size and body size on our inferences (Ives et al. 2007; Silvestro et al. 2015). Error in predictor variables can cause attenuation bias in regression slopes (Hansen and Bartoszek 2012). As an indication of the impact this has on our slope estimates, we calculated the reliability ratio for ordinary least squares regression using an ANOVA on body mass data for each of the allometric regimes we identified with more than 10 species. Dividing the estimated slope by the reliability ratio yields the true slope corrected for attenuation. We found that the median reliability ratio was 0.99, and we therefore conclude that our results are generally robust to slope attenuation bias. Intraspecific variation in bill size may inflate our estimates of the allometric residual variance within regimes. We do not have enough repeat measurements within species to estimate intraspecific variation in bill centroid size measurements from our own data. We therefore turned to published data and found that the intraspecific coefficient of variation in bill size ranges between ~3% and 7% across bird species (Montoya et al. 2018; Rodrigues et al. 2019; Cardona-Salazar et al. 2020; Kennedy et al. 2020; Tsai et al. 2020). Bayou allows the user to specify fixed measurement error estimates to be taken into account in the inference of parameters. We reran bayou chains on the

Muscicapoidea, Falconiformes, Coraciimorphae, and Sylvioidea clades with estimates of intraspecific coefficients of variation of 3%, 5%, and 7%. These clades were chosen as examples because they reflect a range of estimated allometric conservatism. We found that when accounting for a range of possible intraspecific variation, our estimates of stationary variance for these clades are largely congruent with what we estimated in our main analyses that assumed zero measurement error (table s2).

4.3.5 Temporal Trends in Allometric Shifts and the Diversity of Allometric Regimes

We used our estimates of shifts in allometric slope and intercept across the phylogeny to test for trends in the accumulation of allometric disparity (i.e., variation in slopes and intercepts of allometric relationships) through time. For each branch on which we inferred a shift, we took the midpoint of the branch as the point in time when the shift occurred. We restricted our analysis to regimes containing at least 10 extant species. The root regimes for each of our 18 clades were included only if the 95% HPD intervals for either slope or intercept did not overlap with the slope and intercept estimates of the global allometric model across all bird species. We excluded the Strisores root regime as an outlier because we have reason to believe that the model is misspecified for this regime on account of a major difference in bill shape between potoos, oilbirds, and nightjars and the most basal clade of hummingbirds. We first used principal component analysis to generate a space of slopes and intercepts across all regimes. We then inferred an empirical disparity through time curve using the R package dispRity (Guillerme 2018) by calculating the cumulative sum of Euclidean distances from the centroid of the slope-intercept data to each slope-intercept coordinate in principal component space over time. We next generated a null distribution of 1000 disparity through time curves. If shifts have accumulated at a constant rate through time, the probability of recovering a shift in a given time slice should depend on the number of branches in that time slice, with a greater probability of recovering a more recent shift than an ancient one. We therefore pruned the phylogeny to branches subtending at least 10 species and sampled random time slices for the occurrence of our observed shifts in proportion to the number of branches of the phylogeny present in those time slices. We tested for departures from the null distribution of curves in our empirical disparity through time curve using the rank-envelope test (Murrell 2018).

4.4 Results and Discussion

4.4.1 Allometric Diversity

We identified 53 statistically supported shifts in allometric relationships across the bird phylogeny that subtend clades with at least two representative species (Figs. 4.1 and 4.2; Table S1). We also identified many additional shifts on branches supporting a single species (Table S3). Although some of these are likely candidates for lineages that have experienced an allometric shift, such as the sword-billed hummingbird *Ensifera ensifera*, the scythebill *Campylorhamphus trochilirostris*, and the palm cockatoo *Probosciger aterrimus*, other outliers more likely reflect errors in bill size and/or body size data or phylogenetic placement. Although some shifts subtend large and ancient clades with upward of 100 representative species, the majority of shifts subtend young clades with relatively few species. For the 53 shifts we inferred, the median number of sampled species in clades subtended by a shift is 14 and the median shift age is 14 Myr. Some shifts are nested within clades that have themselves shifted from the ancestral allometric relationship within their clade.

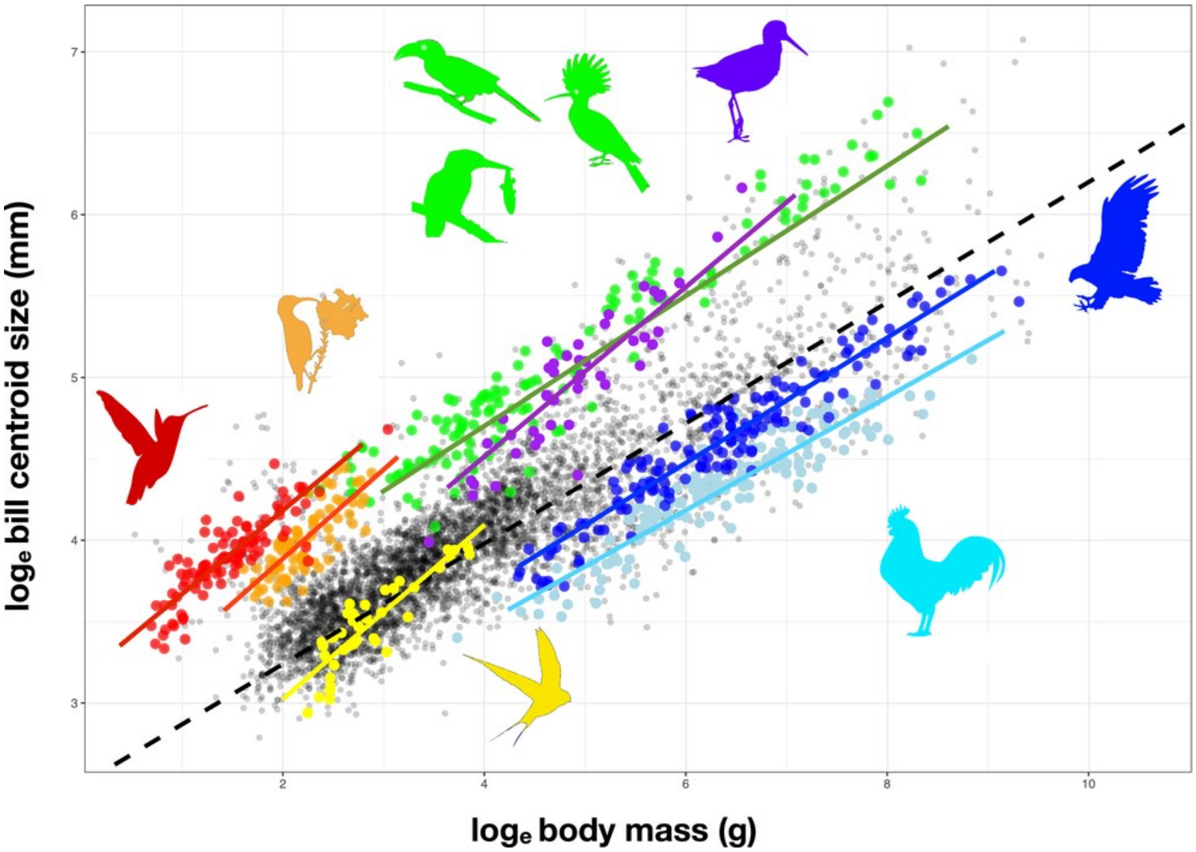

Fig. 4.1 Allometric relationships between beak size and body size illustrated for several clades. Bird silhouettes were obtained from phylopics.org under a creative commons license.

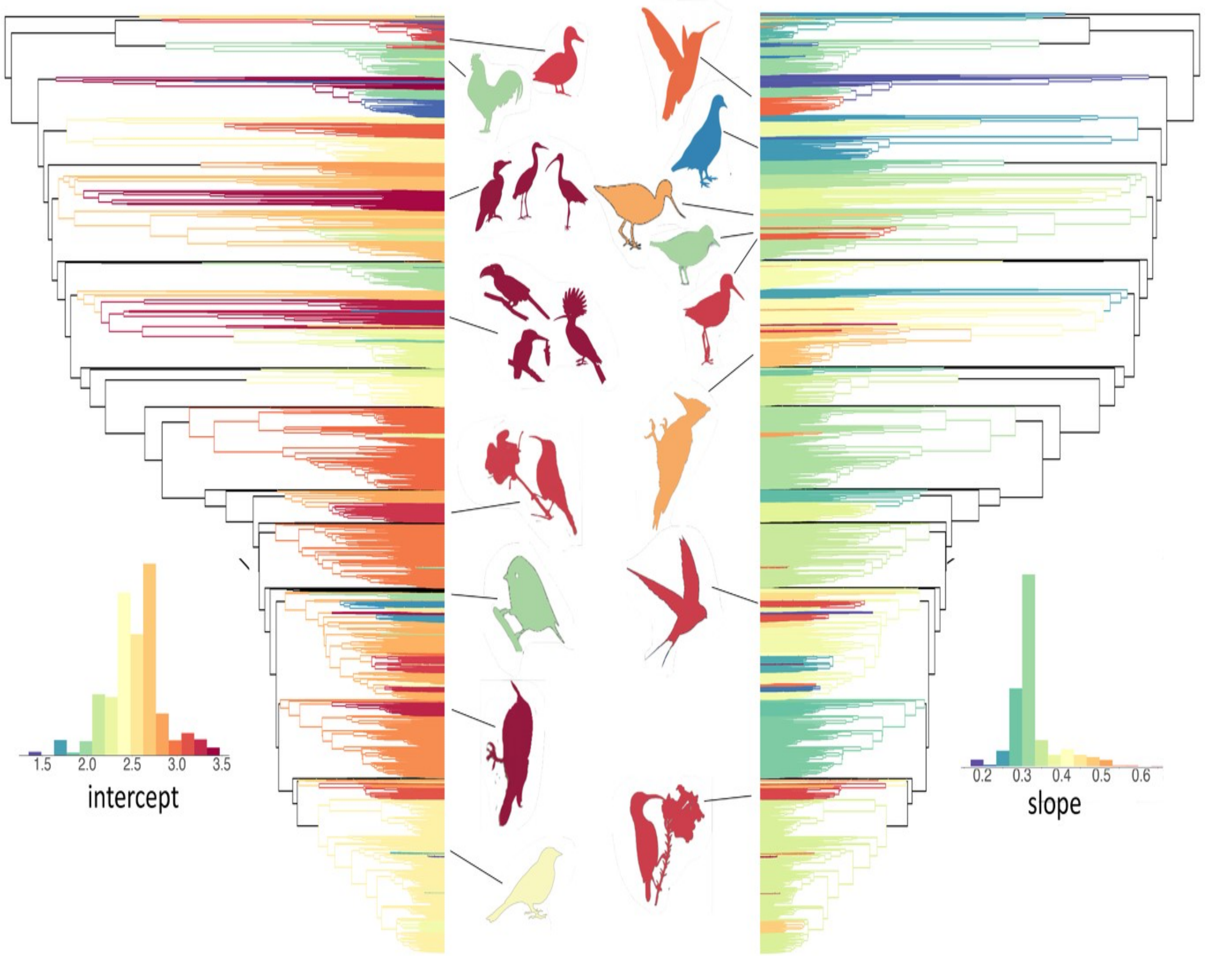

Fig. 4.2 The phylogenetic distribution of allometric intercepts and slopes. Histogram insets show the frequency distributions of parameters across branches of the phylogeny. Bird silhouettes represent the following clades: (left-hand side from top to bottom) ducks and geese; fowls; cormorants, herons, ibises, etc.; hornbills, hoopoes, kingfishers, etc.; honeyeaters; tits; treecreepers, etc.; finches, etc.; (right-hand side from top to bottom) hummingbirds; doves, etc.; oystercatchers and stilts; stints and turnstones; sandpipers; woodpeckers; swallows; sunbirds. Further details on clades can be found in Table S1. Bird silhouettes were obtained from phylopics.org under a creative commons license.

We find examples of several different patterns in the evolution of bill size relative to body size in birds. We find cases of parallelism in allometric relationships consistent with ecological convergence between clades. We find such parallelism between the sandpipers and a clade comprising the stilts, avocets, and oystercatchers, and also between hummingbirds and sunbirds. The most common example of convergence we find is among nectar-feeding birds independently acquiring relatively long bills to extract nectar from flowers. Allometric shifts are usually associated with the acquisition of a specific ecological niche that remains conserved across species, but we also find cases where there has been extensive ecological divergence despite relative conservatism in the allometric relationship between bill size and body size. The most notable examples are the Pelecaniformes (gannets, cormorants, herons, etc.) and the core Coraciimorphae (hornbills, bee eaters, kingfishers, etc.). Finally, we also find examples of evolutionary reversals from extremes of relative bill size to bill morphology more typical of birds as a whole. Notable examples are the sunbird genus *Anthreptes*, the honeyguides among the Piciformes (woodpeckers, toucans, and barbets), and the stints and turnstones among the sandpipers.

The majority of bird lineages evolve around a nearly isometric slope in the relationship between beak size and body size (Fig. 2, histogram insets), so that differences in relative beak size between clades are usually mostly attributable to shifts in intercept. This distribution of slopes is skewed, however, with some clades having strongly positive allometry accounting for large beak size relative to body size in some species. It may be hypothesized that this diversity reflects differences in the underlying developmental trajectories producing variation in adult morphologies. Shifts in intercept may reflect changes in the relative rate of growth of the beak early in development, whereas a steep allometric slope across species may reflect a rapid rate of beak growth relative to body size that is sustained during postnatal development and conserved across closely related species. These hypotheses remain to be tested with comparative ontogenetic data.

4.4.2 Allometric Conservatism

The degree of conservatism within allometric regimes can be quantified using our estimates of stationary variance under an OU model, which predicts the expected residual variance around the slope of the allometric relationship. Our estimates of stationary variance (σ^2) can be expressed as coefficients of variation (cv) using the transformation $cv = \sqrt{e^{\sigma^2} - 1}$. Across the 18 major bird clades we analysed, the median coefficient of variation in bill size for a given body size is 15%. This can be contrasted with a coefficient of variation of 34% under a model assuming all bird species evolve under a single allometric regime. As a further point of comparison, the maximum range in bill size for species of the same body size is roughly between 35% below and 160% above the expected mean, corresponding to the difference in bill size between two species of the same body size, the piping plover *Charadrius melodus* and the rufous-lored kingfisher *Todiramphus winchelli*. There is thus evidence for strong conservatism within allometric regimes.

4.4.3 Temporal Trends in Allometric Shifts and the Diversity of Allometric Regimes

Although the range of bird bill size in relation to body size may have expanded early, allometric diversity across clades has accumulated at a steady rate through time with no evidence that the rate of shifts or the average magnitude of shifts has changed through time (Figs. 4.3 and 4.4). Rather than an early burst of divergence followed by stasis as species pack ever more tightly within existing sets of niches, the pattern we recover suggests that bird lineages have continued to colonize new adaptive zones via allometric shifts up to the present day. The observed cumulative disparity through time curve bends upward toward the present, consistent with the null expectation that more recent shifts have a greater probability of being recovered than more ancient shifts, analogous to the pull of the present in lineage through time plots (Nee et al. 1994).

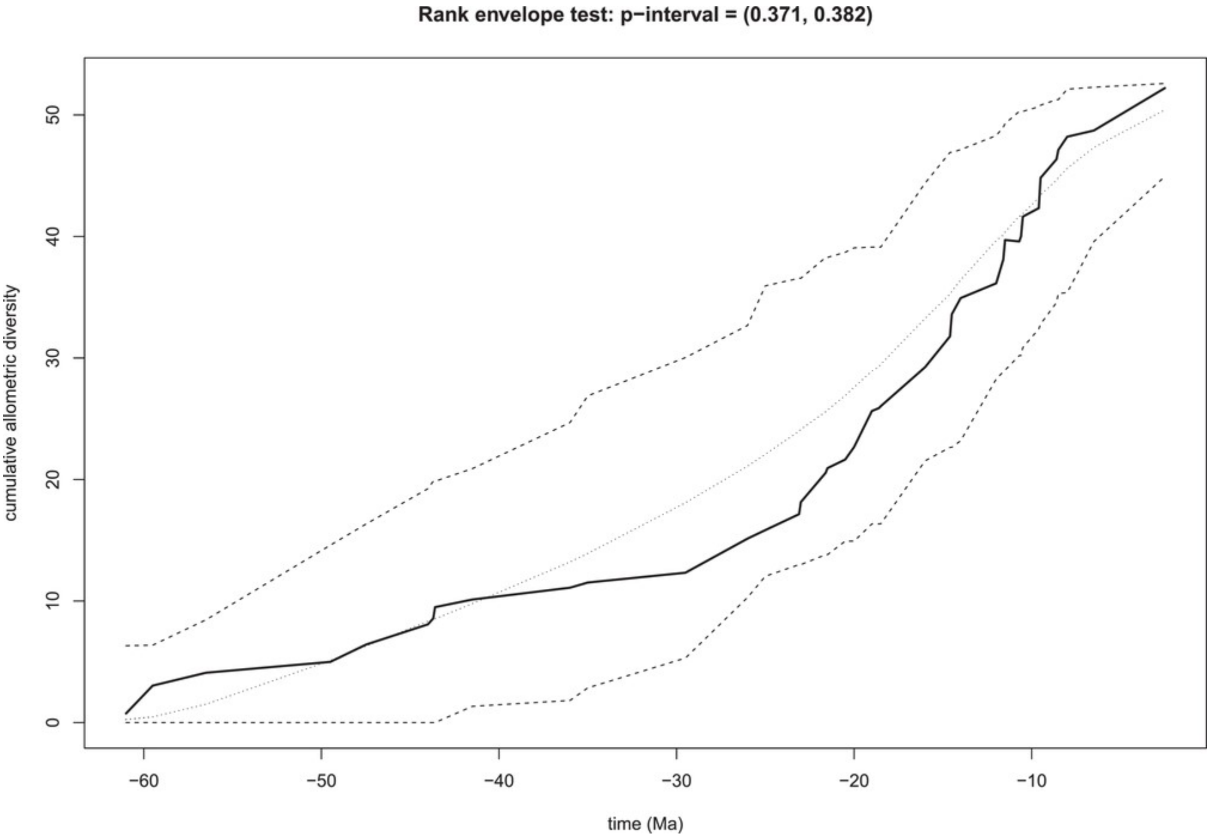

Fig. 4.3 The accumulation of allometric diversity through time measured as the cumulative sum of Euclidean distances of slope-intercept coordinates to the centroid of slope-intercept space. The solid line is the empirical curve of accumulation and the dotted lines represent the upper and lower 95% simulated confidence limits under the null model.

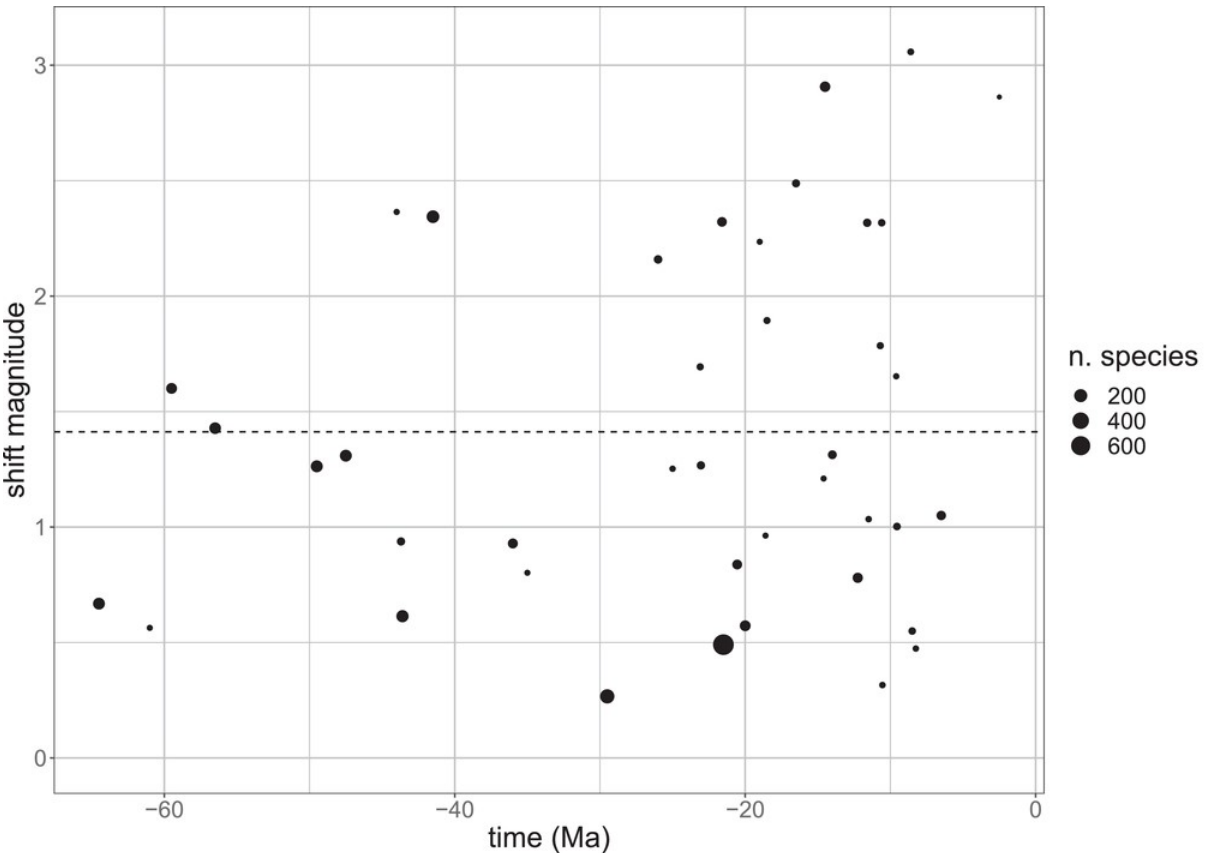

Fig. 4.4 The magnitude of inferred shifts through time measured as the Euclidean distance in slope-intercept space to ancestral regimes. Each point represents a distinct allometric regime and point size is proportional to number of species presently within that regime. The slope of the relationship is nonsignificant ($P > 0.05$).

The number of distinct allometric regimes we can recover is a question of power to detect shifts in allometry. Our power to resolve allometric regimes is limited by the length of time that lineages have had to evolve within distinct regimes, the magnitude of difference in slope and intercept between regimes, and the degree of allometric conservatism within regimes (Ho and Ané 2014; Uyeda and Harmon 2014). Because we use comparative data from living taxa, an important limitation of our work is that we cannot detect ancient shifts in allometry in lineages whose descendants have all become extinct. We are limited to reconstructing the history of allometric shifts that have given rise to the present diversity of bird beaks, which may or may not be representative of the complete history of bird beak evolution in species extinct and extant (Mitchell 2015).

4.4.4 Allometric Conservatism: ecological opportunity vs. genetic-developmental constraints

What are the causes of allometric conservatism over macroevolutionary timescales? Genetic-developmental constraints could be an important driver of conservatism from micro- to macroevolutionary timescales. Potential genetic-developmental constraints on variability include linkage disequilibrium between genes, pleiotropy of genes and new mutations, as well as epistatic interactions promoting developmental canalization by buffering the developing organism from environmental and mutational noise (Walsh and Blows 2009; Svensson et al. 2021). Maintaining robustness in the growth and development of the phenotype is likely a target of stringent selection. Rather than being an impediment to adaptation, however, genetic-developmental constraints may in fact facilitate adaptative evolution if the direction of selection is usually aligned with genetic lines of least resistance (Schluter 1996). Theoretically, a certain degree of pleiotropy is also optimal for evolvability because it presents a bigger target for mutations on which selection can act (Hansen 2003). For these reasons, genetic-developmental constraints may remain conserved across species over macroevolutionary timescales. Although progress has been made in our understanding of the genetic-developmental basis of variation in bird beak size and shape (Grant et al. 2006; Mallarino et al. 2011, 2012), more research is necessary to understand the mechanistic basis of constraints on the independent evolution of the beak (Fritz et al. 2014).

Genetic-developmental constraints alone cannot be a sufficient explanation for macroevolutionary conservatism. We know of several examples of rapid evolution suggesting that genetic-developmental constraints may be readily broken given the right selection pressures. For instance, under artificial selection domesticated pigeons have evolved a striking diversity of beak size and shape (Young et al. 2017). The island radiations of Darwin's finches, Hawaiian honeycreepers, and Madagascan vangas are further examples of rapid adaptive evolution in response to selection (Lovette et al. 2002; Reddy et al. 2012; Navalón et al. 2020). In this study, we have identified several other lineages that have diverged rapidly from their ancestors in the recent past, against a background of allometric conservatism. It is likely that macroevolutionary conservatism is maintained by an interaction between genetic-developmental constraints and limits on ecological opportunity for shifts to new adaptive zones.

4.5 Acknowledgements

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Data used in this study can be accessed on figshare: https://figshare.com/articles/dataset/body_mass_and_beak_size_data_for_the_world_s_birds/16556145.

4.6 References

1. Bouckaert, R., T. G. Vaughan, J. Barido-Sottani, S. Duchêne, M. Fourment, A. Gavryushkina, et al. 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Comput.Biol. 15:1–28.
2. Bright, J. A., J. Marugán-Lobón, S. N. Cobb, and E. J. Rayfield. 2016. The shapes of bird beaks are highly controlled by nondietary factors. Proc. Natl. Acad. Sci. USA 113:5352–5357.
3. Bright, J. A., J. Marugán-Lobón, E. J. Rayfield, and S. N. Cobb. 2019. The multifactorial nature of beak and skull shape evolution in parrots and cockatoos (Psittaciformes). BMC Evol. Biol. 19:1–9.
4. Cardona-Salazar, L. J., Y. A. Benavides-Ossa, Á. M. Vargas-Daza, J. F. Betancurt-Grisales, M. Bohada-Murillo, E. T. Martínez-Sánchez, et al. 2020. A morphological, reproductive, and molt phenology database for 379 bird species from the Colombian Tropical Andes. Ecology 101:10052408.
5. Conner, J. K., I. A. Cooper, R. J. La Rosa, S. G. Pérez, and A. M. Royer. 2014. Patterns of phenotypic correlations among morphological traits across plants and animals. Philos. Trans. R. Soc. B Biol. Sci. 369:20130246.
6. Cooney, C. R., J. A. Bright, E. J. R. Capp, A. M. Chira, E. C. Hughes, C. J. A. Moody, et al. 2017. Mega-evolutionary dynamics of the adaptive radiation of birds. Nature 542:344–347.
7. Cooper, N.,Thomas G. H., Venditti C., Meade A., and Freckleton R. P. 2016. A cautionary note on the use of Ornstein Uhlenbeck models in macroevolutionary studies. Biol. J. Linn. Soc. 118:64–77.
8. Felice, R. N., and A. Goswami. 2018. Developmental origins of mosaic evo- lution in the avian cranium. Proc. Natl. Acad. Sci. USA 115:555–560.
9. Fritz, J. A., J. Brancale, M. Tokita, K. J. Burns, M. B. Hawkins, A. Abzhanov, et al. 2014. Shared developmental programme strongly constrains beak shape diversity in songbirds. Nat. Commun. 5:1–9.
10. Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7:457–472.
11. Grant, P. R., and B. R. Grant. 2006. Evolution of character displacement in Darwin's finches. Science 313:224–226.
12. Grant, P. R., B. R. Grant, and A. Abzhanov. 2006. A developing paradigm for the development of bird beaks. Biol. J. Linn. Soc. 88:17–22.
13. Guillerme, T. 2018. dispRity: a modular R package for measuring disparity. Methods Ecol. Evol. 9:1755–1763.
14. Hansen, T. F. 2003. Is modularity necessary for evolvability? Remarks on the relationship between pleiotropy and evolvability. Biosystems 69:83–94.
15. Hansen, T. F., and K. Bartoszek. 2012. Interpreting the evolutionary regres- sion: the interplay between observational and biological errors in phylogenetic comparative studies. Syst. Biol. 61:413–425.
16. Ho, L. S. T., and C. Ané. 2014. Intrinsic inference difficulties for trait evo- lution with Ornstein-Uhlenbeck models. Methods Ecol. Evol. 5:1133–
17. Houle, D., L. T. Jones, R. Fortune, and J. L. Sztepanacz. 2019. Why does allometry evolve so slowly? Integr. Comp. Biol. 59:1429–1440.
18. Huxley, J. 1932. Problems of relative growth. Dover, Mineola, NY.
19. Ives, A. R., P. E. Midford, and T. Garland. 2007. Within-species variation and measurement error in phylogenetic comparative methods. Syst. Biol. 56:252–270.
20. Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. Nature 491:444–448.
21. Kennedy, J. D., P. Z. Marki, J. Fjeldså, and C. Rahbek. 2020. The association between morphological and ecological characters across a global passerine radiation. J. Anim. Ecol. 89:1094–1108.
22. Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution. 33:402–416
23. Lovette, I. J., E. Bermingham, and R. E. Ricldefs. 2002. Clade-specific mor- phological diversification and adaptive radiation in Hawaiian songbirds. Proc. R. Soc. B Biol. Sci. 269:37–42.
24. Mallarino, R., P. R. Grant, B. R. Grant, A. Herrel, W. P. Kuo, and A. Abzhanov. 2011. Two developmental modules establish 3D beak-shape variation in Darwin's finches. Proc. Natl. Acad. Sci. USA 108:4057– 4062.
25. Mallarino, R., O. Campàs, J. A. Fritz, K. J. Burns, O. G. Weeks, M. P. Bren- ner, et al. 2012. Closely related bird species demonstrate flexibility be- tween beak morphology and underlying developmental programs. Proc. Natl. Acad. Sci. USA 109:16222–16227.

26. Mitchell, J. S. 2015. Extant-only comparative methods fail to recover the disparity preserved in the bird fossil record. Evolution 69:2414– 2424.

27. Montoya, P., M. A. Gonzalez, E. A. Tenorio, J. P. López-Ordóñez, A. Pinto Gómez, and D. Cueva. 2018. A morphological database for 606 Colom- bian bird species. Ecology 99:1693.

28. Murrell, D. J. 2018. A global envelope test to detect non-random bursts of trait evolution. Methods Ecol. Evol. 9:1739–1748.

29. Myhrvold, N. P., E. Baldridge, B. Chan, D. Sivam, D. L. Freeman, and S. K. M. Ernest. 2015. An amniote life-history database to perform com- parative analyses with birds, mammals, and reptiles. Ecology 96:3109– 3000.

30. Navalón, G., J. Marugán-Lobón, J. A. Bright, C. R. Cooney, and E. J. Ray- field. 2020. The consequences of craniofacial integration for the adap- tive radiations of Darwin's finches and Hawaiian honeycreepers. Nat. Ecol. Evol. 4:270–278.

31. Nee, S., R. M. May, and P. H. Harvey. 1994. The reconstructed evolutionary process. Philos. Trans. R. Soc. B Biol. Sci. 344:305–311.

32. Outomuro, D., and F. Johansson. 2017. A potential pitfall in studies of biological shape: does size matter? J. Anim. Ecol. 86:1447– 1457.

33. Pigot, A., C. Sheard, E. T. Miller, T. P. Bregman, B. G. Freeman, U. Roll, et al. 2020. Macroevolutionary convergence connects morphological form to ecological function in birds. Nature Ecol. Evol. 4:230–239

34. Prum, R. O., J. S. Berv, A. Dornburg, D. J. Field, J. P. Townsend, E. M. Lemmon, et al. 2015. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. Nature 526:569–573.

35. Reddy, S., A. Driskell, D. L. Rabosky, S. J. Hackett, and T. S. Schulenberg. 2012. Diversification and the adaptive radiation of the vangas of Mada- gascar. Proc. R. Soc. B Biol. Sci. 279:2062–2071.

36. Rodrigues, R. C., É. Hasui, J. C. Assis, J. C. C. Pena, R. L. Muylaert, V. R. Tonetti, et al. 2019. ATLANTIC BIRD TRAITS: a data set of bird mor- phological traits from the Atlantic forests of South America. Ecology 100:1–2.

37. Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

38. Shatkovska, O. V., and M. Ghazali. 2020. Integration of skeletal traits in some passerines: impact (or the lack thereof) of body mass, phylogeny, diet and habitat. J. Anat. 236:274–287.

39. Silvestro, D., A. Kostikova, G. Litsios, P. B. Pearman, and N. Salamin. 2015. Measurement errors should always be incorporated in phyloge- netic comparative analysis. Methods Ecol. Evol. 6:340–346.

40. Simpson, G. G. 1944. Tempo and mode in evolution. National Academy of Sciences, Washington, D.C.

41. ———. 1953. The major features of evolution. Columbia Univ. Press, New York.

42. Skandalis, D. A., P. S. Segre, J. W. Bahlman, D. J. E. Groom, K. C. Welch, C. C. Witt, et al. 2017. The biomechanical origin of extreme wing allome- try in hummingbirds. Nat. Commun. 8:1–8.

43. Svensson, E. I., S. J. Arnold, R. Bürger, K. Csilléry, J. Draghi, J. M. Henshaw, et al. 2021. Correlational selection in the age of genomics. Nat. Ecol. Evol. 5:562–573.

44. Tsai,P.Y.,C.J.Ko,C.Hsieh,Y.T.Su,Y.J.Lu,R.S.Lin,etal.2020.Atrait dataset for Taiwan's breeding birds. Biodivers. Data J. 8:e49735.

45. Uyeda, J. C., and L. J. Harmon. 2014. A novel Bayesian method for inferring and interpreting the dynamics of adaptive landscapes from phylogenetic comparative data. Syst. Biol. 63:902–918.

46. Uyeda, J. C., M. W. Pennell, E. T. Miller, R. Maia, and C. R. McClain. 2017. The evolution of energetic scaling across the vertebrate tree of life. Am. Nat. 190:185–199.

47. Van Den Elzen, R., and H. Nemeschkal. 2007. The impact of body mass on morphological integration in avian skeletons. Bonner Zool. Beiträge 56:25–35.

48. Voje, K. L., T. F. Hansen, C. K. Egset, G. H. Bolstad, and C. Pélabon. 2014. Allometric constraints and the evolution of allometry. Evolution 68:866–885.

49. Walsh, B., and M. W. Blows. 2009. Abundant Genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. Annu. Rev. Ecol. Evol. Syst. 40:41–59.

50. Young, N. M., M. Linde-Medina, J. W. Fondon, B. Hallgrímsson, and R. S. Marcucio. 2017. Craniofacial diversification in the domestic pigeon and the evolution of the avian skull. Nat. Ecol. Evol. 1:1–8.

4.7 Supplementary Materials

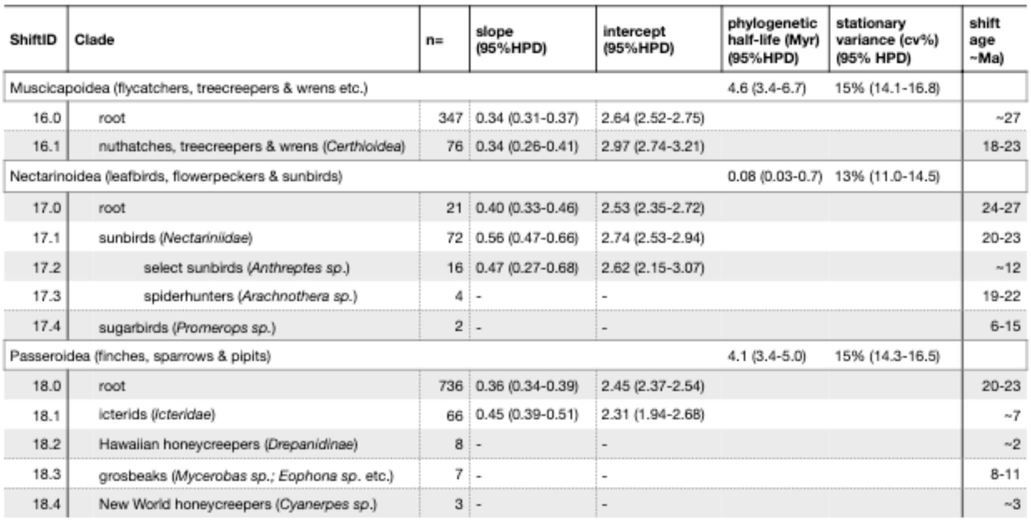
Table 1. Allometric Shifts and Parameter Estimates

ShiftID	**Clade**	**n=**	**slope (95%HPD)**	**intercept (95%HPD)**	**phylogenetic half-life (Myr) (95%HPD)**	**stationary variance (cv%) (95% HPD)**	**shift age ~(Ma)**
Palaeognathae (ratites & tinamous)					0.17 (0.05-17)	8% (5.8-12.1)	
1.0	root	13	0.31 (0.28-0.33)	2.50 (2.32-2.69)			50-72
1.1	kiwis (*Apteryx sp.*)	4	-	-			4-26
1.2	select tinamous (*Crypturellus sp.*)	5	-	-			~23
Anseriformes (ducks & geese)					1.4 (0.8-3.3)	15% (12.4-17.8)	
2.0	root	89	0.32 (0.27-0.37)	2.79 (2.45-3.12)			46-54
2.1	swans & geese (*Anserinae*)	19	0.44 (0.37-0.52)	1.49 (0.82-2.12)			~9
2.2	teals & shovelers (*Spatula sp.*)	10	0.50 (0.38-0.63)	1.71 (0.80-2.60)			~3
2.3	sheldgeese (*Chloephaga sp.*)	4	-	-			~3
Galliformes (fowls)					0.6 (0.3-2.0)	14% (12.4-15.5)	
3.0	root	150	0.34 (0.32-0.37)	2.14 (1.98-2.30)			45-54
3.1	guans & curassows (*Craciidae*)	23	0.28 (0.21-0.35)	2.76 (2.23-3.26)			9-37
3.2	grouse (*Tetraoninae*)	15	0.30 (0.22-0.38)	2.21 (1.65-2.75)			~9
Strisores (nightjars, swifts & hummingbirds)					45 (26-165)	42% (32.1-83.0)	
4.0	root	51	0.23 (0.13-0.33)	3.36 (2.86-3.81)			64-67
4.1	coquettes & brilliants	51	0.34 (0.21-0.48)	2.12 (1.23-2.97)			~15
4.2	emeralds, mountain gems & bees	96	0.51 (0.44-0.59)	1.72 (0.86-2.58)			~15
4.3	swifts (*Apodidae*)	14	0.23 (0.05-0.42)	1.86 (0.96-2.75)			41-47
Columbaves (cuckoos, bustards & doves)					6.2 (4.3-10.4)	15% (13.7-17.2)	
5.0	root	146	0.31 (0.28-0.34)	2.44 (2.28-2.62)			62-67
5.1	cuckoos (*Cuculidae*)	81	0.37 (0.32-0.42)	2.70 (2.47-2.93)			~36
5.2	sandgrouse (*Pteroclidae*)	13	0.24 (0.10-0.37)	2.35 (1.60-3.14)			17-53
Gruiformes (rails, crakes & cranes)					8.6 (4.6-33)	28% (22.3-40.2)	
6.0	root	74	0.34 (0.30-0.39)	2.60 (2.31-2.89)			40-65
Aequorlitornithes (gulls, sandpipers, herons etc.)					20 (15-31)	31% (26.8-36.5)	
7.0	root	266	0.35 (0.32-0.38)	2.56 (2.36-2.77)			~64
7.1	stilts, avocets & oystercatchers (*Recurvirostridae + Haematopidae*)	15	0.46 (0.35-0.56)	2.51 (1.59-3.43)			22-28
7.2	curlews, godwits & other sandpipers (*Scolopacidae*)	44	0.52 (0.44-0.60)	2.21 (1.48-2.89)			22-30
7.3	stints & turnstones (*Eroliinae*)	22	0.36 (0.25-0.47)	2.49 (1.62-3.34)			17-20
7.4	gannets, cormorants & herons (*Pelecaniformes*)	109	0.37 (0.32-0.41)	3.27 (2.85-3.71)			~60
Accipitriformes (vultures, hawks & eagles)					0.2 (0.08-1.2)	13% (11.9-15.1)	
8.0	root	149	0.39 (0.37-0.41)	2.15 (2.02-2.29)			39-56
8.1	New World vultures (*Cathartidae*)	4	-	-			6-56
8.2	serpent eagles (*Spilornis sp.*)	5	-	-			~5

Table 1. Allometric Shifts and Parameter Estimates

ShiftID	**Clade**	**n=**	**slope (95%HPD)**	**intercept (95%HPD)**	**phylogenetic half-life (Myr) (95%HPD)**	**stationary variance (cv%) (95% HPD)**	**shift age ~(Ma)**
Coraciimorphae (hornbills, bee eaters, kingfishers etc.)					17 (13-27)	20% (17.5-23.6)	
9.0	root (owls, trogons & mousebirds)	45	0.32 (0.24-0.39)	2.57 (2.17-2.94)			~61
9.1	core coraciimorphae (hornbills, bee eaters, kingfishers etc.)	137	0.39 (0.35-0.43)	3.18 (2.97-3.43)			~57
9.2	rollers (*Coraciidae*)	8	-	-			15-43
9.3	jacamars (*Galbulidae*)	8	-	-			22-34
9.4	woodpeckers & barbets (*Piciformes*)	182	0.46 (0.42-0.49)	2.30 (1.97-2.54)			34-49
9.5	honeyguides (*Indicatoridae*)	13	0.39 (0.32-0.45)	2.07 (1.50-2.60)			14-23
9.6	toucans (*Ramphastidae*)	13	0.57 (0.49-0.65)	2.32 (1.42-3.21)			13-16
Falconiformes (falcons)					0.1 (0.04-1.1)	10% (7.9-12.2)	
10.0	root	39	0.34 (0.30-0.38)	2.27 (2.04-2.49)			29-58
10.1	caracaras (*Caracarinae*)	8	-	-			8-17
Psittaciformes (parrots)					4.1 (2.7-8.0)	16% (13.6-19.1)	
11.0	root	161	0.36 (0.33-0.39)	2.40 (2.24-2.57)			32-55
Suboscines (ovenbirds, cotingas & manakins etc.)					10 (7.6-14)	18% (16.8-21.0)	
12.0	root	424	0.35 (0.32-0.38)	2.67 (2.55-2.79)			41-46
12.1	woodcreepers (*Dendrocolaptinae*)	23	0.49 (0.38-0.60)	2.28 (1.57-2.97)			9-12
Meliphagoidea (pardalotes, fairy wrens & honeyeaters etc.)					8.7 (5.4-19)	19% (16.8-24.6)	
13.0	root	70	0.33 (0.28-0.39)	2.59 (2.37-2.79)			39-46
13.1	honeyeaters (*Meliphagidae*)	107	0.37 (0.32-0.42)	2.85 (2.59-3.12)			18-22
Corvoidea (crows, cuckooshrikes & birds of paradise etc.)					7.8 (5.7-11.7)	17% (15.6-19.6)	
14.0	root	338	0.36 (0.33-0.39)	2.66 (2.54-2.78)			~31
14.1	longrunners (*Orthonychidae*)	3	-	-			8-24
14.2	longbills (*Toxorhamphus sp.*)	2	-	-			3-11
14.3	riflebirds (*Ptiloris sp.*)	5	-	-			~5
14.4	*Drepanornis sp.*	2	-	-			~5
14.5	*Epimachus sp.*	2	-	-			~4
Sylvoidea (tits, larks & warblers etc.)					0.11 (0.04-0.5)	11% (10.5-11.8)	
15.0	root	262	0.37 (0.35-0.39)	2.58 (2.52-2.63)			~30
15.1	tits & chickadees (*Paridae*)	40	0.43 (0.35-0.52)	2.15 (1.93-2.38)			21-25
15.2	larks (*Alaudidae*)	36	0.55 (0.46-0.63)	1.98 (1.69-2.27)			~16
15.3	select larks (*Galerida sp.;Spizocorys sp.* etc.)	27	0.37 (0.27-0.48)	2.46 (2.08-2.80)			9-12
15.4	acrocephalid warblers (*Acrocephalidae*)	28	0.42 (0.32-0.52)	2.58 (2.31-2.85)			~9
15.5	prinias, cisticolas & apalises (*Cisticolidae*)	87	0.32 (0.26-0.38)	2.80 (2.65-2.94)			~13
15.6	tailorbirds (*Orthotomus sp.*)	5	-	-			~8
15.7	crombecs & longbills (*Macrosphenidae*)	13	0.22 (0.11-0.33)	3.18 (2.88-3.49)			~20
15.8	swallows & martins (*Hirundinidae*)	41	0.53 (0.45-0.60)	1.98 (1.76-2.20)			10-13
15.9	select bulbuls (*Phyllastrephus sp.*)	16	0.39 (0.23-0.53)	2.67 (2.20-3.20)			9-12
15.10	old world babblers (*Timaliidae*)	14	0.51 (0.41-0.61)	2.34 (2.04-2.66)			8-11
15.11	jungle babblers (*Pellorneidae*)	29	0.29 (0.20-0.39)	2.94 (2.61-3.27)			~10
15.12	bushtits & long-tailed tit (*Aegithalidae*)	5	-	-			10-14

Table 1. Allometric Shifts and Parameter Estimates



ShiftID	Clade	n=	slope (95%HPD)	intercept (95%HPD)	phylogenetic half-life (Myr) (95%HPD)	stationary variance (cv%) (95% HPD)	shift age ~Ma)
Muscicapoidea (flycatchers, treecreepers & wrens etc.)					4.6 (3.4-6.7)	15% (14.1-16.8)	
16.0	root	347	0.34 (0.31-0.37)	2.64 (2.52-2.75)			~27
16.1	nuthatches, treecreepers & wrens (*Certhioidea*)	76	0.34 (0.26-0.41)	2.97 (2.74-3.21)			18-23
Nectarinoidea (leafbirds, flowerpeckers & sunbirds)					0.08 (0.03-0.7)	13% (11.0-14.5)	
17.0	root	21	0.40 (0.33-0.46)	2.53 (2.35-2.72)			24-27
17.1	sunbirds (*Nectariniidae*)	72	0.56 (0.47-0.66)	2.74 (2.53-2.94)			20-23
17.2	select sunbirds (*Anthreptes sp.*)	16	0.47 (0.27-0.68)	2.62 (2.15-3.07)			~12
17.3	spiderhunters (*Arachnothera sp.*)	4	-	-			19-22
17.4	sugarbirds (*Promerops sp.*)	2	-	-			6-15
Passeroidea (finches, sparrows & pipits)					4.1 (3.4-5.0)	15% (14.3-16.5)	
18.0	root	736	0.36 (0.34-0.39)	2.45 (2.37-2.54)			20-23
18.1	icterids (*Icteridae*)	66	0.45 (0.39-0.51)	2.31 (1.94-2.68)			~7
18.2	Hawaiian honeycreepers (*Drepanidinae*)	8	-	-			~2
18.3	grosbeaks (*Mycerobas sp.; Eophona sp.* etc.)	7	-	-			8-11
18.4	New World honeycreepers (*Cyanerpes sp.*)	3	-	-			~3

Table S2. Intraspecific Error Sensitivity Analyses (median coefficient of variation of stationary OU variance and 95%HPD).

Clade	3%	5%	7%
Muscicapoidea	15.1 (13.8-17)	14.9 (13.3-17.7)	14.8 (12.6-20.3)
Falconiformes	9.1 (7.2-11.8)	8.1 (6-11.1)	6.3 (3.1-9.8)
Coraciimorphae	20.2 (17.3-25.4)	21.1 (17.2-30.6)	22.5 (17-92.2)
Sylvoidea	10.7 (10.1-11.5)	10.7 (10.1-11.5)	9.2 (8-12)

S3 Outlier Taxa

Cyanochen cyanoptera
Malacorhynchus membranaceus
Pauxi pauxi
Macrodipteryx longipennis
Ensifera ensifera
Ramphomicron microrhynchum
Oreonympha nobilis
Rhodopis vesper
Chalcostigma heteropogon
Myrtis fanny
Musophaga violacea
Cacomantis merulinus
Monias benschi
Ptilinopus perousi
Larus bulleri
Sterna vittata
Larus glaucescens
Ixobrychus sinensis
Ixobrychus minutus
Chondrohierax uncinatus
Strix aluco
Trogon viridis
Trogon chionurus
Aceros cassidix
Bycanistes fistulator
Indicator xanthonotus
Sphyrapicus ruber
Dendrocopos cathpharius
Dendrocopos leucotos
Dendrocopos obsoletus
Piculus flavigula
Lybius rolleti
Calyptorhynchus funereus
Probosciger aterrimus
Drymornis bridgesii
Campylorhamphus trochilirostris
Manorina melanocephala
Urolestes melanoleucus
Lophorina superba
Seleucidis melanoleucus
Muscicapa ussheri
Ficedula mugimaki
Cyornis superbus
Panurus biarmicus
Zosterops albogularis
Foudia eminentissima
Ploceus temporalis
Loxioides bailleui
Serinus syriacus
Serinus mennelli
Serinus buchanani
Plectrophenax nivalis
Arremon aurantiirostris
Arremon crassirostris
Nesospiza wilkinsi
Sporophila bouvronides

Chapter 5

The Evolution of the Traplining Pollinator Role in Hummingbirds: Is specialisation an evolutionary dead end?

5.1 Abstract

Trapliners are pollinators that visit widely dispersed flowers along circuitous foraging routes. The evolution of traplining in hummingbirds is thought to entail morphological specialization through the reciprocal coevolution of longer bills with the long-tubed flowers of widely dispersed plant species. Specialization, such as that exhibited by traplining hummingbirds, is often viewed as both irreversible and an evolutionary dead end. We tested these predictions in a macroevolutionary framework. Specifically, we assessed the relationship between beak morphology and foraging and tested whether transitions to traplining are irreversible and lead to lower rates of diversification as predicted by the hypothesis that specialization is an evolutionary dead end. We find that there have been multiple independent transitions to traplining across the hummingbird phylogeny, but reversals have been rare or incomplete at best. Multiple independent lineages of trapliners have become morphologically specialized, convergently evolving relatively large bills for their body size. Traplining is not an evolutionary dead end however, since trapliners continue to give rise to new traplining species at a rate comparable to non-trapliners.

5.2 Introduction

For plants that are widely dispersed across a landscape, there is a premium in attracting high-fidelity long-range pollinators and excluding low-fidelity short-range pollinators (Torres-

Vanegas et al., 2019; Ohashi & Thomson, 2009; Sargent & Otto, 2006; Ghazoul, 2005; Stiles, 1975; 1978; Linhart, 1973). Trapliners are pollinators that visit widely dispersed flowers along circuitous foraging routes. Widely dispersed plant species may gain an advantage from having adaptations in floral morphology, such as nectar spurs or long corolla tubes, that allow access to rich nectar rewards for traplining pollinators while barring access to non-trapliners (Warren & Diaz, 2001; Borrell, 2005; Johnson & Steiner, 1997; Tavares, Freitas & Gaglianone, 2016). For specialization as a trapliner to be profitable, plants must offer adequate rewards to compensate for the energetic cost of travelling between widely dispersed flowers and the opportunity cost of ignoring flowers of other species in the same vicinity (Charnov, 1976). Trapliners should in turn evolve morphological adaptations, such as long bills, that allow them to access the nectar of such flowers (Temeles, 1996; Temeles et al., 2009; Wolf, Hainsworth & Stiles, 1972; Maglianesi, Bohning-Gaese, Schleuning, 2015; Maglianesi et al., 2014). Through coevolution with the flowers of the various species they pollinate, trapliners may therefore become more morphologically and ecologically specialized than their non-traplining counterparts. This hypothesis on the coevolution between guilds of widely dispersed flowers and traplining pollinators inspires several macroevolutionary predictions, which we test in this study of hummingbirds.

The most basic of these predictions is that the evolution of traplining should entail convergent morphological specialization. Specifically, we addressed the prediction that through coevolution with the flowers they pollinate, trapliners should evolve relatively large bills for their body size and a higher wing surface area relative to body size (Feinsinger & Colwell, 1978; Feinsinger & Chaplin, 1975). In evolving morphological specialization to a subset of flowers, trapliners may experience more rapid rates of morphological evolution than the average non-trapliner due to directional selection driven by reciprocal coevolution between flowers and pollinators (Pauw, Stofberg & Waterman, 2009; Nuismer, Jordano & Bascompte, 2013). In particular, as flowers evolve longer corolla tubes, hummingbirds should quickly evolve longer bills. Directional selection for longer bills may also lead to lineages of trapliners breaking ancestral allometric constraints on bill evolution, so that clades of trapliners have weaker evolutionary correlations between bill and body size.

Ecological specialization on a subset of resources could be hypothesized to be an 'evolutionary dead end' in the sense that evolutionary reversals back to a generalized ecology are rare and specialized species rarely give rise to new specialized species (Futuyama & Moreno, 1988; Carscadden et al., 2020). The evolution of an increasingly specialized morphology in hummingbirds to exploit specific sets of flowers may make subsequent reversal to a generalist niche more unlikely. While an adaptive ramp may be available for hummingbirds to become increasingly morphologically specialized through gradual coevolution with flowers, reversals may be hindered by an absence of a gradual adaptive ramp in the reverse direction due to competition with short-billed hummingbird species for short-tubed flowers. An ecologically specialized hummingbird may also be more vulnerable to extinction than a generalist and may have reduced potential to spawn new ecologically distinct species. This effect has been found in some groups of organisms (Raia et al., 2016; McGee et al., 2015). Hence, clades of specialist hummingbirds may have lower rates of diversification than generalist clades. On the other hand, clades of specialist trapliners may be able to diversify in the specific flowers on which they feed, supporting high rates of diversification, the opposite of what the evolutionary dead ends hypothesis would predict. Evidence in the literature for specialization being an evolutionary dead end is currently mixed (Day, Hua & Bromham, 2016; Vamosi, Armbruster & Renner, 2014). Here, we test these ideas on hummingbirds using phylogenetic comparative methods to characterize diversification and rates of morphological evolution in relation to evolutionary transitions in foraging ecology.

5.3 Methods

We took three-dimensional (3D) scans of the entire bill and linear measurements of bill length, bill width, bill depth, wing length and tail length from one male museum specimen for each of 289 species at the Ornithological Collection of the Natural History Museum in Tring (UK). Using data from Graham et al. (2012), we estimate that intraspecific variance in body mass is only approximately 1.3% of interspecific variance in our data and intraspecific variance in bill length is only approximately 1.1% of interspecific variance, so intraspecific variation in morphological traits is unlikely to impact our results on this scale of analysis. We 3D-scanned and landmarked bills of specimens as described in Cooney et al. (2017). There were four fixed landmarks and three semi-landmark curves of 25 points each. We used the R package geomorph (Adams & Otarola-Castillo, 2013) to perform landmark alignments and extract principal components (PCs) of shape variation as well as bill centroid size (mm), an overall measure of bill size defined as the square root of the sum of squared Euclidean distances from the centroid to each of the landmarks. We retained the first three PCs describing greater than 95% of the variation in shape. We obtained data on the mean body mass of each species in grams from Wilman et al. (2014). All morphological measurements were $\log_e$ transformed.

We were able to classify 238 hummingbird species (approx. 80% of all genera) as either trapliners (70), territorial (104) or opportunists (64). We also performed sensitivity analyses where opportunists and territorial species were classed as non-trapliners. We obtained information on the foraging behaviour of hummingbird species from the '*Handbook of the Birds of the World*' (del Hoyo et al., 1999), the Cornell Lab of Ornithology's '*Birds of the World*' online database (www.birdsoftheworld.org) (Billerman et al., 2020) and the '*Hummingbirds of North America*' (Johnsgard, 1997). There can be strong sexual dimorphism within hummingbird species in both morphology and foraging behaviour [Linhart, 1973; Temeles et al., 2009, Temeles & Kress, 2003; Berns & Adams, 2013). Since we obtained morphological data for male museum specimens, species were classified according to the foraging behaviour of males wherever sex-specific information was available. There are 12 species in our assembled dataset where foraging behaviour is described for both sexes, and there is sexual dimorphism in six of these. The terms 'traplining' and 'territorial' are regularly used as a dichotomy to describe hummingbird foraging behaviour in the literature. We also considered descriptions such as 'visiting dispersed flowers' or 'following circuitous foraging routes' as further support for classifying a species as a trapliner and descriptions such as 'feeding on clumps of flowers' or 'displaying aggression towards other hummingbirds' as further support for classifying a species as territorial. Species we classified as opportunists are those that are described as 'facultatively territorial' or displaying territorial behaviour seasonally or in some geographical locations but not others. Some hummingbird species are described as 'filchers', sneaking into the territories of other hummingbirds to feed on flowers. Since many of these species are also described as 'facultatively territorial', they were classified as opportunists. We acknowledge the potential for error in foraging classifications. However, the inclusion of species with uncertain classification into the intermediate opportunist category should increase our power to detect differences between species that are confidently classed as trapliners and those confidently classed as territorial.

We used two alternative published phylogenies in our comparative analyses (Jetz et al., 2012; McGuire et al., 2014). Trees from Jetz et al. (2012) (available from www.birdtree.org) are based on genetic sequence data plus taxonomic imputation for 299 species, while trees from ref. McGuire et al. (2014) (available from https://tree.opentreeoflife.org) are based on genetic data only for 291 species. We constructed maximum clade credibility trees from phylogenetic posterior distributions using TreeAnnotator (Bouckaert et al., 2019). Taxonomic labels for ecological and morphological data were matched to the two phylogenies with taxonomic synonym information from the Cornell Lab of Ornithology's '*Birds of the World*' online database (www.birdsoftheworld.org) (Billerman et al., 2020).

5.3.1 (i) Does the evolution of traplining entail convergent morphological specialization?

We used random forest classification models (Liaw & Wiener, 2002; Pigot et al., 2020) to test whether differences in foraging behaviour are associated with differences in the dimensions of morphological traits. Random forest models use sets of decision trees to classify items according to multiple variables and have an advantage in accounting for potentially complex multi-dimensional relationships between predictor and response variables. First, we performed a PC analysis (PCA) on all morphological traits combined and used the PCs as predictors of foraging behaviour. We also repeated the analysis using phylogenetic PCA. In a second model, we performed a separate PCA on bill centroid size and body mass alone and used these PCs as predictors, as these traits are expected to be most closely associated with foraging behaviour. PC1 accounted for 68% of the variation and PC2 accounted for the remainder. Because this reduced model had the same predictive power as the full model (see §3), we focused on just these two traits in the rest of our analyses. We tuned forest size and number of variables to consider at each split by trying different values and seeing which maximized classification accuracy. Following initial tuning of forest size and number of variables to consider at each split, we used fivefold cross-validation to estimate classification accuracy. To generate a null expectation of classification accuracy based on observed phylogenetic similarity among species, we simulated the evolution of traits randomly under the Brownian motion model of evolution 1000 times using the fastBM function of the R package phytools (Revell, 2012) on rate-scaled trees inferred using BayesTraits v3 with default settings (Pagel & Meade, 2004) (http://www.evolution.rdg.ac.uk/; see below) and used these randomly simulated traits as predictors of foraging behaviour. We generated simulated data for bill centroid size and body mass independently. As a complementary analysis, we used phylogenetic generalized least squares (PGLS) in the R package caper (Orme, 2018) to test for differences in the slopes and intercepts of allometric relationships between bill size and body size among trapliners, territorialists and opportunists.

5.3.2 (ii) Does the evolution of traplining entail higher rates of morphological evolution and weaker evolutionary correlation between bill size and body size?

We used the R package ratematrix (Caetano & Harmon, 2017; May & Moore, 2020) to test for an association between foraging behaviour and rates of morphological evolution in bill size and body size, as well as differences in the evolutionary correlation between bill size and body size. For each discrete state, ratematrix estimates a variance–covariance matrix for morphological traits under a correlated Brownian motion model of evolution. We supplied ratematrix with 1000 stochastic character mappings of the evolutionary history of foraging behaviour on our phylogenies generated using the make.simmap function of phytools (Revell, 2012). We set normal priors on the phylogenetic root states of bill size and body size with means and standard deviations equal to those estimated from the data. We set a lognormal prior on the evolutionary variances of both traits with a mean of 0.1 and standard deviation of 1.5. For the correlation between traits, we set a uniform prior. We ran four independent Markov chain Monte Carlo (MCMC) chains in parallel for 20 million generations each with a burnin proportion of 0.25. We repeated analyses using 'trapliner' and 'non-trapliner' classifications.

Determining whether differences in rates of morphological evolution are associated with particular character states can be complicated by background rate variation. We therefore used the MuSSCRat model (May & Moore, 2020), implemented in RevBayes (Hohna et al., 2016) (https://revbayes.github.io/), as an additional test of our hypothesis. This allows us to test whether character states are associated with different rates of evolution beyond what would be expected from random background variation alone. We note, however, that the MuSSCRat code currently available only allows for overall rates in trait evolution, and not correlations between traits, to vary between examined states. We ran four chains for 100 000 generations each with a burnin of 10 000 generations. All priors and settings were the defaults except for the prior on the number of expected number of transitions which we set to 20 based on a prior judgement of how many transitions in foraging ecology there appear to have been in the tree.

To further identify and visualize how rates of morphological evolution vary across the phylogeny independent of foraging behaviour, we used BayesTraits to fit a variable rates model of correlated evolution between bill size and body size. Two chains were run in parallel for 110 million generations each with a burnin of 10 million generations. All other priors and settings were the defaults.

For all analyses involving MCMC sampling, we visualized traces and posterior distributions using Tracer (Rambaut et al., 2018). We checked that the effective sample size for parameters was greater than 200 and that Gelman and Rubin's R diagnostic among chains was less than 1.05.

5.3.3 (iii) Is traplining an evolutionary dead end?

We tested two predictions of the hypothesis that traplining is an evolutionary dead end. First, we fitted models of evolutionary transitions in foraging behaviour using the fitDiscrete function in the R package geiger (Pennell et al., 2014). From fitting a full model in which all transition rates between states were estimated, we found that the transition rates from traplining to territorialism and from opportunism to traplining were very close to zero. We therefore fitted a reduced model in which these transition rates were fixed to zero and compared the full and reduced models using the Akaike information criterion (AIC). We used this to test for irreversibility in transitions.

Second, we tested whether traplining is associated with lower net diversification rates. Whether a discrete state is associated with differences in rates of speciation or extinction across a phylogeny can be complicated by background rate variation (Rabosky & Goldberg, 2015). We therefore used the R package SecSSE (Herrera-Alsina, Van Els & Etienne, 2019) to jointly estimate transition rates and test for state-dependent diversification. We tested models in which rates of speciation and extinction differ between the three foraging states examined against models in which speciation and extinction rates differ between three hidden states and models with constant background rates. We tested models with one, four and six independent transition rates between states. To avoid the possibility of getting stuck in a local likelihood optimum, we ran five independent repetitions of the likelihood maximization algorithm with different starting points as follows: (1) speciation and extinction rates set to their maximum-likelihood estimates (ML) from a simple birth–death model + transition rates set to 1/5 of speciation rates; (2) ML speciation rates double + ½ transition rates; (3) ½ ML speciation rates + double transition rates; (4) double ML extinction rates + ½ transition rates and (5) ½ ML extinction rates + double transition rates. We set num_cycles = Inf and optimmethod = ‘subplex’. We selected the maximum of the five estimated likelihoods to compare models. We compared models using AIC scores. We assumed the sampling fraction of phylogenies to be 0.88 for all examined states based on the proportion of taxonomically recognized species (338) represented in the phylogeny. We repeated analyses using the trapliner versus non-trapliner classification of foraging behaviour.

5.4 Results

5.4.1 Does the evolution of traplining entail convergent morphological specialization?

The random forest classification model predicted foraging behaviour from PCs of all morphological traits with an accuracy of 61% (σ = 6%) or 58% if using phylogenetic PCA. However, PCs of body mass and bill centroid size alone are sufficient as predictors to achieve a classification accuracy of 60% (σ = 5%). This can be contrasted with the classification accuracy achieved when the random forest model is applied to data simulated under the assumption that morphology evolves independently of foraging behaviour (classification accuracy: 41%; 95% CI = 34–50%). Opportunists might be expected to be intermediate between trapliners and territorialists, bringing down the overall classification accuracy. As expected, when only considering trapliners and territorialists, the overall classification accuracy increases to 75% (σ= 11%). The classification accuracy remains 75% when using phylogenetic PCA.

There is a significant difference in the slope of the allometric relationship between bill size and body size between trapliners, opportunists and territorialists (PGLS: $F_{2,207} = 5.71$, $p = 0.004$). The significance of this result is robust to the exclusion of the evolutionary outlier *Ensifera ensifera*, the sword-billed hummingbird which has an extremely long bill. Between trapliners and opportunists, trapliners tend to have the largest extremes of relative bill size, although the predicted phylogenetic regressions are very close in the two groups. It could be hypothesized that opportunists are therefore ecologically adapted to traplining since they are at least facultatively trapliners. This is not inconsistent with our hypotheses. Trapliners, and opportunists, tend to have relatively larger bills for their body size than territorialists (fig. 5.1). To a large extent, this trend is driven by species of the hermit hummingbird clade (Phaethorninae). Trapliners of the inca clade (*Coeligena*) have independently converged on a hermit-like morphology, as have multiple isolated lineages of trapliners: e.g. *E. ensifera*, *Androdon aequatorialis*, *Doryfera* species, *Polytmus* species and *Myrtis fannyi*. Members of the traplining *Chlorostilbon* and *Lophornis* clades, however, fall within the morphological range of non-traplining hummingbirds.

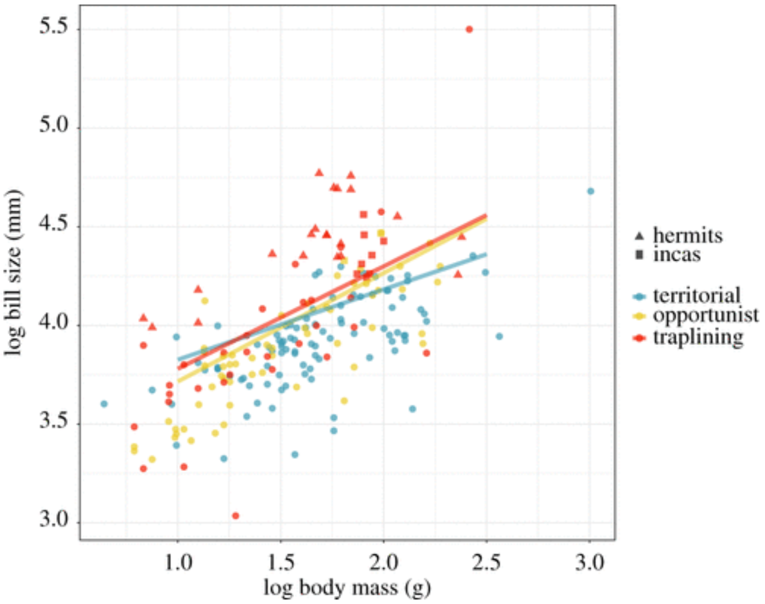
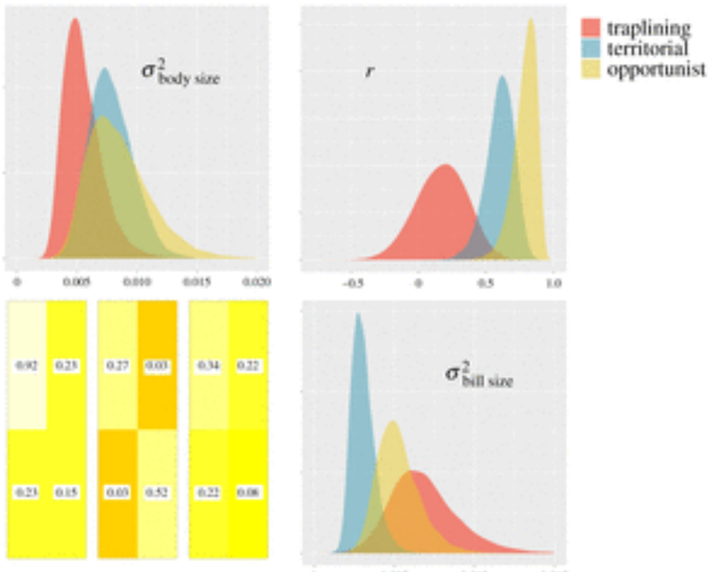


Fig. 5.1 Allometry in bill centroid size (mm) and body size (g) of hummingbirds (PGLS): territorialists: log(bill size) = 0.36 × log(body mass) + 3.47; opportunists: log(bill size) = 0.55 × log(body mass) + 3.17; trapliners: log(bill size) = 0.52 × log(body mass) + 3.26.

5.4.2 Does the evolution of traplining entail higher rates of morphological evolution and weaker evolutionary correlation between bill size and body size?

Inferred differences among traplining, territorialist and opportunist lineages in estimated average rates of bill size and body size evolution, and their evolutionary correlation inferred using ratematrix, are sensitive to the phylogeny on which analyses are performed. (The results of all pairwise comparisons can be found in these figures: fig. 5.2; supplementary material, figures S1 and S2.) We used the results of the BayesTraits analyses of variable rates of trait evolution to identify possible causes of the inconsistent results across trees. This showed that the two main differences between the phylogenies are the presence of *Hylonympha macrocerca* and the greater upshift in rates of evolution within the bee hummingbird clade in the Jetz *et al*. (2012) phylogeny (supplementary material, figure S3). When we repeat the ratematrix analysis with the evolutionary outlier species *H. macrocerca* pruned from the Jetz *et al*. (2012) phylogeny, there is no longer a significant difference in rates of body size evolution between trapliners, territorialists and opportunists (overlap in Bayesian posterior distributions greater than 5%). However, even when the Jetz *et al.* (2012) phylogeny is pruned to have only species that are also present in the McGuire *et al.* (2014) phylogeny, there is still a discrepancy between the two sets of analyses. Only when *H. macrocerca* and all members of the bee hummingbird clade are pruned from the Jetz *et al.* (2012) phylogeny are the results congruent with analyses on the McGuire *et al.* (2014) phylogeny. Given these facts and the significant overlap in posterior distributions for parameters, we conclude that there is no definitive evidence for differences in the average rate of bill size and body size evolution, or the evolutionary correlation between them, for trapliners, territorialists and opportunists. This remains true when considering the binary classification of 'trapliners' and 'non-trapliners' (supplementary material, figure S2). This conclusion is supported by analysis with the MuSSCRat model (supplementary material, figure S4) which indicates that rates of evolution are variable across the hummingbird phylogeny but are unrelated to foraging behaviour.

(*a*) McGuire



(*b*) Jetz

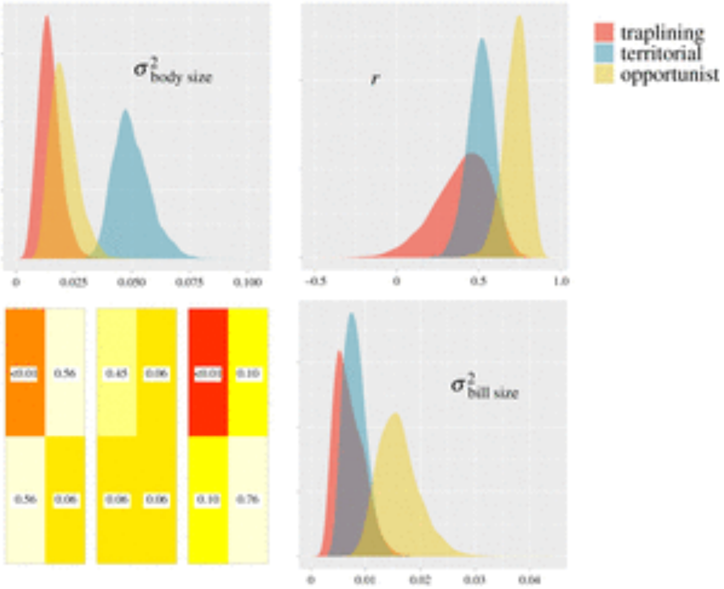


Fig. 5.2. Analyses of state-dependent correlated trait evolution with ratematrix. Ratematrix analyses were repeated on the McGuire *et al.* [38] phylogeny (*a*) and the Jetz *et al.* [37] phylogeny (*b*). In each panel, the posterior distributions of evolutionary rate (σ^2) and correlation (r) parameters are compared for different ecological regimes: traplining in red, opportunism in yellow and territorialism in blue. The upper left panel is for rates of body size evolution, the bottom right for rates of bill size evolution and the top right for their evolutionary correlation. The proportion of overlap between posterior distributions in pairwise comparisons between regimes is indicated in the bottom left panel, with darker colours suggesting a more definite difference. Each rectangle in the bottom left panel follows the structure of the 2 × 2 evolutionary variance–covariance matrix of bill size and body size evolution. From left to right, the three pairwise comparisons are: opportunist × territorial, opportunist × traplining and territorial × traplining. Numbers within rectangles are proportion overlaps between distributions.

5.4.3 Is traplining an evolutionary dead end?

Transitions to an exclusively traplining lifestyle are relatively rare in hummingbirds compared to transitions between territorialism and opportunism (fig. 5.3; supplementary material, figure S5). Reversals from traplining to territorialism are rarer still. We found that a model in which the transition rate from traplining to territorialism was fixed to zero had a better fit than a model in which all transition rate parameters were free to vary (ΔAIC = 4; supplementary material, table S6), suggesting that such transitions are mostly irreversible. Multiple traplining lineages have transitioned to opportunism however, becoming at least facultatively territorial.

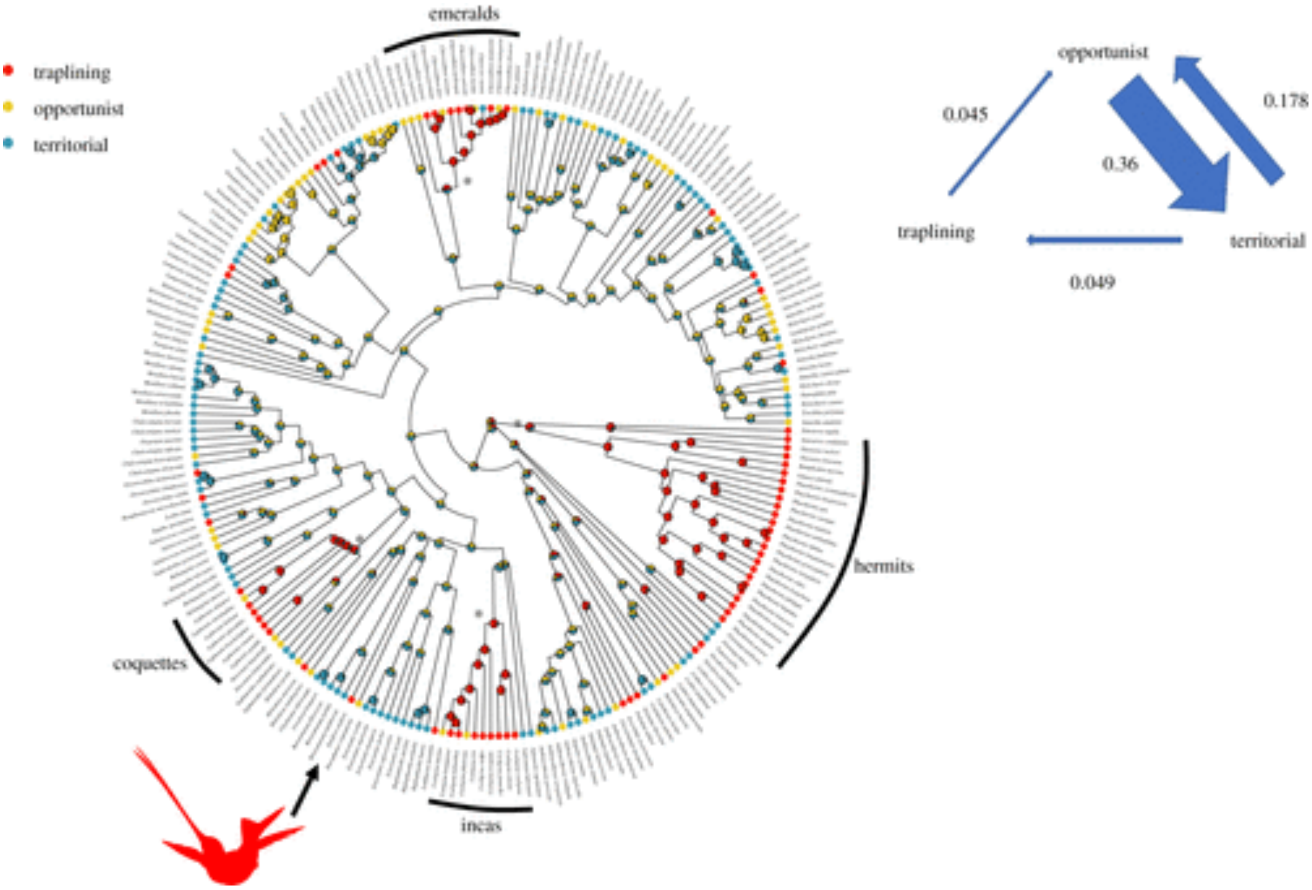


Figure 5.3. The evolution of hummingbird foraging ecology and transition rates between states. The asterisks point to locations on the phylogeny where there was likely an origin of a major traplining clade. Pie charts represent probabilities for ancestral foraging strategies. The red silhouette shows *Ensifera ensifera*.

Our estimation of ancestral states on the hummingbird phylogeny identifies several non-sister clades of traplining and opportunist species with a likely traplining ancestor: the hermits (Phaethorninae), with approximately 37 species found mostly in the tropical lowlands of South America; the incas (*Coeligena*), an Andean clade with approximately 11 species; the coquettes (*Lophornis*), with approximately 10 species inhabiting South and Central America and emeralds of the genus *Chlorostilbon,* with approximately 18 species found in South and Central America and the Caribbean. Additionally, we identify multiple isolated lineages of trapliners represented by only one or two species.

Based on the results of the SecSSE analysis, there is no evidence to suggest that rates of speciation are generally any different for traplining, territorial or opportunist clades (supplementary material, tables S7–S9). The best-supported models among the models we analysed indicate variation in speciation rates among hidden states unrelated to our foraging classifications (ΔAIC $\gg$ 4).

5.5 Discussion

Specialization may be expected to result in convergent morphological specialization, elevated rates of morphological evolution and evolutionary dead ends. Our findings challenge these assumptions. Although multiple lineages of trapliners have independently become morphologically specialized to feed on long-tubed flowers, many other lineages of trapliners remain morphologically unspecialized. While some trapliners such as the sword-billed hummingbird (*E. ensifera*) have experienced greatly elevated rates of morphological evolution, other trapliners have not. Finally, though some clades of trapliners have low rates of diversification, other clades have relatively high rates of diversification for hummingbirds.

Multiple transitions from territorialism to traplining have taken place in the course of hummingbird evolution (fig. 5.3), while reversals from traplining to territorialism are rare or incomplete in the sense that trapliners only ever become facultatively territorial opportunists. Traplining is essentially a behavioural characteristic of a species' foraging ecology. Behavioural traits are thought to have intrinsically high adaptive plasticity within species and evolutionary lability across species (Blomberg, Garland & Ives, 2003). We infer relatively high rates of transition between lineages that are territorial and lineages that are facultatively territorial opportunists, but once traplining evolves in a lineage, it tends to be conserved. Lineages that evolve to become true trapliners may also quickly become adaptively specialized to a niche as trapliners. Ecological competition among species, mediated by morphological traits, is an important process in hummingbird community assembly (Graham et al., 2012; Lessard et al., 2015; Brown & Bowers, 1985). It is possible that ecological competition with other hummingbird species may prevent traplining hummingbirds from re-adapting to life as territorialists. Traplining is not an evolutionary dead end however, as in our best-supported model traplining species give rise to new species at a rate comparable to non-trapliners.

Hummingbird diversification is characterized by disparity in rates among major clades (McGuire et al., 2014), but that disparity is unrelated to foraging behaviour. The clade of bee hummingbirds in particular has experienced elevated rates of diversification and stands out as one with a generally elevated rate of evolution in bill size and body size in our analyses. It may be that the ecological niches that bee hummingbirds have occupied as uniquely small hummingbirds have spurred both their diversification and high rates of morphological evolution. We are cautious in this interpretation because elevated rates of trait evolution can arise as an artefact of a combination of trait measurement errors, the effects of short branch lengths and phylogenetic error (Rabosky & Goldberg, 2015; Rabsoky, 2015; Cooney & Thomas, 2021). While we have no reason to assume that phylogenetic error is a more severe problem for bee hummingbirds than any other clade, we cannot rule out the possibility that trait measurement error has some effect. This is because bee hummingbirds are among the smallest hummingbirds and even if absolute measurement error is similar among clades, the potential for higher proportional error could disproportionately elevate our measures of trait evolution for this clade.

While we do see evidence for repeated convergent evolution of large bills relative to body size in trapliners, we do not find conclusive evidence for an association between foraging behaviour and rates of bill and body size evolution or the evolutionary correlation between them. One may not necessarily expect to detect elevated rates of trait evolution in sets of living species if adaptive peak shifts which gave rise to the current disparity in morphological traits happened long ago (Harmon et al, 2010). Adaptive peak shifts may entail punctuational breaks in patterns of trait evolution though equilibrium quickly re-establishes itself. Traplining lineages that have only recently experienced high rates of morphological evolution due to coevolution with flowers may be poorly represented among modern species.

Our macroevolutionary hypotheses necessarily make assumptions about the chain of causation from the spatial distribution of plant species within habitats to the coevolution among the traits of plants and pollinators to the evolution of ecological specialization. Empirical evidence provides support for these assumptions. Widely dispersed plants benefit from being pollinated by traplining hummingbirds (Torres-Vanegas et al., 2019; Ghazoul, 2005; Stiles, 1975; 1978; Linhart, 1973), and the bill length of hummingbirds is correlated with the corolla length of the flowers they visit and with ecological specialization (Charnov, 1976; Maglianesi et al., 2014; Sonne et al., 2019; Tinoco et al., 2017). However, there is some evidence to challenge the assumption that traplining hummingbirds are always more ecologically specialized than territorialists. Traplining hermits visit more plant species than other hummingbirds in at least one community (Cotton, 1998). Morphological specialization need not result in ecological specialization if there are minimal trade-offs involved in exploiting a wide range of flowers when resources are abundant, while maintaining adaptations to exploit a narrower range of flowers at times of resource scarcity and high competition (Robinson & Wilson, 1998; Weinstein & Graham, 2017). Failure of our predictions to hold generally across all clades of trapliners may be a reflection of the fact that these assumptions on the chain of causation are not always met.

In conclusion, we found that the relationship among the evolution of traplining, morphological specialization, rates of morphological evolution and diversification is complex, and it does not lead to simple deterministic outcomes. In a broad comparative study of ecological adaptation, it is difficult to account for complex lineage-specific factors balancing the costs and benefits of evolution towards morphological and ecological specialization. This could be addressed with more detailed field studies on the foraging ecology of different clades of traplining hummingbirds, as well as trapliners in other groups of pollinators.

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Code and data necessary to reproduce the analyses presented in this paper are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.q83bk3jhp [**64**].

5.7 References

1. Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An r package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, *4*(4), 393–399. https://doi.org/10.1111/2041-210X.12035
2. Berns, C. M., & Adams, D. C. (2013). Becoming Different But Staying Alike: Patterns of Sexual Size and Shape Dimorphism in Bills of Hummingbirds. *Evolutionary Biology*, *40*(2), 246–260. https://doi.org/10.1007/s11692-012-9206-3
3. Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*(4), 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

4. Borrell, B. J. (2005). Long tongues and loose niches: Evolution of euglossine bees and their nectar flowers. *Biotropica*, *37*(4), 664–669. https://doi.org/10.1111/j.1744-7429.2005.00084.x
5. Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., de Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), 1–28. https://doi.org/10.1371/journal.pcbi.1006650
6. Brown, J. H., & Bowers, M. a. (1985). Community Organization in Hummingbirds: Relationships between Morphology and Ecology. *The Auk*, *102*(2), 251–269. https://doi.org/10.2307/4086767
7. Caetano, D. S., & Harmon, L. J. (2017). ratematrix: An R package for studying evolutionary integration among several traits on phylogenetic trees. *Methods in Ecology and Evolution*, *8*(12), 1920–1927. https://doi.org/10.1111/2041-210X.12826
8. Caetano, D. S., & Harmon, L. J. (2018). Estimating correlated rates of trait evolution with uncertainty. *Systematic Biology*, *68*(3), 412–429. https://doi.org/10.1093/sysbio/syy067
9. Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., Livingstone, S. W., & Wiens, J. J. (2020). Niche Breadth: Causes and Consequences for Ecology, Evolution, and Conservation. *The Quarterly Review of Biology*, *95*(3), 179–214. https://doi.org/10.1086/710388
10. Charnov, E. (1976). Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, *752*(4), 739–752.
11. Cooney, C. R., Bright, J. A., Capp, E. J. R., Chira, A. M., Hughes, E. C., Moody, C. J. A., Nouri, L. O., Varley, Z. K., & Thomas, G. H. (2017). Mega-evolutionary dynamics of the adaptive radiation of birds. *Nature*, *542*(7641), 344–347. https://doi.org/10.1038/nature21074
12. Cooney, C. R., & Thomas, G. H. (2021). Heterogeneous relationships between rates of speciation and body size evolution across vertebrate clades. *Nature Ecology and Evolution*, *5*(1), 101–110. https://doi.org/10.1038/s41559-020-01321-y
13. Cotton, P. A. (1998). Coevolution in an Amazonian hummingbird-plant community. *Ibis*, *140*(4), 639–646. https://doi.org/10.1111/j.1474-919x.1998.tb04709.x
14. Day, E. H., Hua, X., & Bromham, L. (2016). Is specialization an evolutionary dead end? Testing for differences in speciation, extinction and trait transition rates across diverse phylogenies of specialists and generalists. *Journal of Evolutionary Biology*, *29*(6), 1257–1267. https://doi.org/10.1111/jeb.12867
15. del Hoyo. (1999). *Handbook of the Birds of the World V5: Barn Owls to Hummingbirds* (J. del Hoyo, A. Elliott, J. Sargatal, & N. J. Collar, Eds.). Lynx Edicions.
16. Feinsinger, P., & Colwell, R. K. (1978). Community organization among neotropical nectar-feeding birds. *Integrative and Comparative Biology*, *18*(4), 779–795. https://doi.org/10.1093/icb/18.4.779
17. Feinsinger, P., & Chaplin, S. (1975). On the Relationship between Wing Disc Loading and Foraging Strategy in Hummingbirds. *American Naturalist*, *109*(966), 217–224.
18. Futuyma, D. J., & Moreno, G. (1988). The Evolution of Ecological Specialization. *Annual Review of Ecology and Systematics*, *19*(1988), 207–233.
19. Ghazoul, J. (2005). Pollen and seed dispersal among dispersed plants. *Biological Reviews of the Cambridge Philosophical Society*, *80*(3), 413–443. https://doi.org/10.1017/S1464793105006731
20. Graham, C. H., Parra, J. L., Tinoco, B. A., Stiles, F. G., & McGuire, J. A. (2012). Untangling the influence of ecological and evolutionary factors on trait variation across hummingbird assemblages. *Ecology*, *93*(8 SPEC. ISSUE), 99–111. https://doi.org/10.1890/11-0493.1
21. Harmon, L. J., Losos, J. B., Jonathan Davies, T., Gillespie, R. G., Gittleman, J. L., Bryan Jennings, W., Kozak, K. H., McPeek, M. A., Moreno-Roark, F., Near, T. J., Purvis, A., Ricklefs, R. E., Schluter, D., Schulte II, J. A., Seehausen, O., Sidlauskas, B. L., Torres-Carvajal, O., Weir, J. T., & Mooers, A. Ø. (2010). Early bursts of body size and shape evolution are rare in comparative data. *Evolution*. https://doi.org/10.1111/j.1558-5646.2010.01025.x
22. Herrera-Alsina, L., van Els, P., & Etienne, R. S. (2019). Detecting the Dependence of Diversification on Multiple Traits from Phylogenetic Trees and Trait Data. *Systematic Biology*, *68*(2), 317–328. https://doi.org/10.1093/sysbio/syy057
23. Höhna, S., Landis, M. J., Heath, T. A., Boussau, B., Lartillot, N., Moore, B. R., Huelsenbeck, J. P., & Ronquist, F. (2016). RevBayes: Bayesian Phylogenetic Inference Using Graphical Models and an Interactive Model-Specification Language. *Systematic Biology*, *65*(4), 726–736. https://doi.org/10.1093/sysbio/syw021
24. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature*, *491*(7424), 444–448. https://doi.org/10.1038/nature11631
25. Johnsgard, P. A. (1997). *The Hummingbirds of North America*. Smithsonian Institution Press.

26. Johnson, S. D., & Steiner, K. E. (1997). Long-tongued fly pollination and evolution of floral spur length in the Disa draconis complex (Orchidaceae). *Evolution*, *51*(1), 45–53. https://doi.org/10.1111/j.1558-5646.1997.tb02387.x
27. Lessard, J. P., Weinstein, B. G., Borregaard, M. K., Marske, K. A., Martin, D. R., McGuire, J. A., Parra, J. L., Rahbek, C., & Graham, C. H. (2015). Process-based species pools reveal the hidden signature of biotic interactions amid the influence of temperature filtering. *American Naturalist*, *187*(1), 75–88. https://doi.org/10.1086/684128
28. Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R News*, *2*(3), 18–22.
29. Linhart, Y. B. (1973). Ecological and Behavioral Determinants of Pollen Dispersal in Hummingbird-Pollinated Heliconia. *The American Naturalist*, *107*(956), 511–523. https://doi.org/10.1086/282854
30. Maglianesi, M. A., BlüThgen, N., BöHning-Gaese, K., & Schleuning, M. (2014). Morphological traits determine specialization and resource use in plant-hummingbird networks in the neotropics. *Ecology*, *95*(12), 3325–3334. https://doi.org/10.1890/13-2261.1
31. May, M. R., & Moore, B. R. (2020). A Bayesian Approach for Inferring the Impact of a Discrete Character on Rates of Continuous-Character Evolution in the Presence of Background-Rate Variation. *Systematic Biology*, *69*(3), 530–544. https://doi.org/10.1093/sysbio/syz069
32. McGee, M. D., Borstein, S. R., Neches, R. Y., Buescher, H. H., Seehausen, O., & Wainwright, P. C. (2015). A pharyngeal jaw evolutionary innovation facilitated extinction in Lake Victoria cichlids. *Science*, *350*(6264), 1077–1079. https://doi.org/10.1126/science.aab0800
33. McGuire, J. A., Witt, C. C., Remsen, J. v., Corl, A., Rabosky, D. L., Altshuler, D. L., & Dudley, R. (2014). Molecular phylogenetics and the diversification of hummingbirds. *Current Biology*, *24*(8), 910–916. https://doi.org/10.1016/j.cub.2014.03.016
34. Nuismer, S. L., Jordano, P., & Bascompte, J. (2013). Coevolution and the architecture of mutualistic networks. *Evolution*, *67*(2), 338–354. https://doi.org/10.1111/j.1558-5646.2012.01801.x
35. Ohashi, K., & Thomson, J. D. (2009). Trapline foraging by pollinators: Its ontogeny, economics and possible consequences for plants. *Annals of Botany*, *103*(9), 1365–1378. https://doi.org/10.1093/aob/mcp088
36. Pagel, M., & Meade, A. (2004). A Phylogenetic Mixture Model for Detecting Pattern-Heterogeneity in Gene Sequence or Character-State Data. *Systematic Biology*, *53*(4), 571–581. https://doi.org/10.1080/10635150490468675
37. Pauw, A., Stofberg, J., & Waterman, R. J. (2009). Flies and flowers in darwin's race. *Evolution*, *63*(1), 268–279. https://doi.org/10.1111/j.1558-5646.2008.00547.x
38. Pennell, M. W., Eastman, J. M., Slater, G. J., Brown, J. W., Uyeda, J. C., Fitzjohn, R. G., Alfaro, M. E., & Harmon, L. J. (2014). Geiger v2.0: An expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics*, *30*(15), 2216–2218. https://doi.org/10.1093/bioinformatics/btu181
39. Pigot, A. L., Sheard, C., Miller, E. T., Bregman, T. P., Freeman, B. G., Roll, U., Seddon, N., Trisos, C. H., Weeks, B. C., & Tobias, J. A. (2020). Macroevolutionary convergence connects morphological form to ecological function in birds. In *Nature Ecology and Evolution* (Vol. 4, Issue 2, pp. 230–239). https://doi.org/10.1038/s41559-019-1070-4
40. Rabosky, D. L. (2015). No substitute for real data: A cautionary note on the use of phylogenies from birth-death polytomy resolvers for downstream comparative analyses. *Evolution*, *69*(12), 3207–3216. https://doi.org/10.1111/evo.12817
41. Rabosky, D. L., & Goldberg, E. E. (2015). Model inadequacy and mistaken inferences of trait-dependent speciation. *Systematic Biology*, *64*(2), 340–355. https://doi.org/10.1093/sysbio/syu131
42. Raia, P., Carotenuto, F., Mondanaro, A., Castiglione, S., Passaro, F., Saggese, F., Melchionna, M., Serio, C., Alessio, L., Silvestro, D., & Fortelius, M. (2016). Progress to extinction: Increased specialisation causes the demise of animal clades. *Scientific Reports*, *6*(0316), 1–10. https://doi.org/10.1038/srep30965
43. Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032
44. Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x
45. Robinson, B. W., & Wilson, D. S. (1998). Optimal foraging, specialization, and a solution to Liem's paradox. *American Naturalist*, *151*(3), 223–235. https://doi.org/10.1086/286113
46. Rodewald, P., Billerman, S., & Keeney, B. (2020). *Birds of the World*.
47. Sargent, R. D., & Otto, S. P. (2006). The role of local species abundance in the evolution of pollinator attraction in flowering plants. *American Naturalist*, *167*(1), 67–80. https://doi.org/10.1086/498433
48. Sonne, J., Zanata, T. B., Martín González, A. M., Cumbicus Torres, N. L., Fjeldså, J., Colwell, R. K., Tinoco, B. A., Rahbek, C., & Dalsgaard, B. (2019). The distributions of morphologically specialized

hummingbirds coincide with floral trait matching across an Andean elevational gradient. *Biotropica*, *51*(2), 205–218. https://doi.org/10.1111/btp.12637

49. Stiles, F. G. (1978). Ecological and evolutionary implications of bird pollination. *American Zoologist*, *18*(4), 715–727. https://doi.org/10.1093/icb/18.4.715
50. Stiles, G. (1975). Ecology , Flowering Phenology , and Hummingbird Pollination of Some Costa Rican Heliconia Species. *Ecology*, *56*(2), 285–301.
51. Tavares, D. C., Freitas, L., & Gaglianone, M. C. (2016). Nectar volume is positively correlated with flower size in hummingbird-visited flowers in the Brazilian Atlantic Forest. *Journal of Tropical Ecology*, *32*(4), 335–339. https://doi.org/10.1017/S0266467416000250
52. Temeles, E. J., Koulouris, C. R., Sander, S. E., & Kress, W. J. (2009). Effect of flower shape and size on foraging performance and trade-offs in a tropical hummingbird. *Ecology*, *90*(5), 1147–1161. https://doi.org/10.1890/08-0695.1
53. Temeles, E. J. (1996). A new dimension to hummingbird - Flower relationships. *Oecologia*, *105*(4), 517–523. https://doi.org/10.1007/BF00330015
54. Tinoco, B. A., Graham, C. H., Aguilar, J. M., & Schleuning, M. (2017). Effects of hummingbird morphology on specialization in pollination networks vary with resource availability. *Oikos*, *126*(1), 52–60. https://doi.org/10.1111/oik.02998
55. Torres-Vanegas, F., Hadley, A. S., Kormann, U. G., Jones, F. A., Betts, M. G., & Wagner, H. H. (2019). The Landscape Genetic Signature of Pollination by Trapliners: Evidence From the Tropical Herb, Heliconia tortuosa. *Frontiers in Genetics*, *10*(December), 1–12. https://doi.org/10.3389/fgene.2019.01206
56. Vamosi, J. C., Armbruster, W., & Renner, S. S. (2014). Evolutionary ecology of specialization: Insights from phylogenetic analysis. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1795). https://doi.org/10.1098/rspb.2014.2004
57. Warren, J., & Diaz, A. (2001). A two-pollinator model for the evolution of floral complexity. *Evolutionary Ecology*, *15*(3), 157–166. https://doi.org/10.1023/A:1014826507699
58. Weinstein, B. G., & Graham, C. H. (2017). Persistent bill and corolla matching despite shifting temporal resources in tropical hummingbird-plant interactions. *Ecology Letters*, *20*(3), 326–335. https://doi.org/10.1111/ele.12730
59. Wilman, H., Belmaker, J., Simpson, J., de la Rosa, C., Rivadeneira, M. M., & Jetz, W. (2014). EltonTraits 1.0: Species-level foraging attributes of the world's birds and mammals. *Ecology*, *95*(7), 2027–2027. https://doi.org/10.1890/13-1917.1
60. Wolf, L. L., Reed Hainsworth, F., & Gary Stiles, F. (1972). Energetics of foraging: Rate and efficiency of nectar extraction by hummingbirds. *Science*, *176*(4041), 1351–1352. https://doi.org/10.1126/science.176.4041.1351

5.8 Supplementary Materials

Fig S1. Results of ratematrix sensitivity analyses. Each block of three rectangles summarises the results of a different sensitivity analysis. In the left column are analyses based on the McGuire et al. (2014) phylogeny and in the right column are results based on the Jetz et al. (2012) phylogeny. Each rectangle within a block of three represents a pairwise comparison between the posterior distributions of evolutionary variance-covariance matrices under different ecological regimes (opportunist v territorial, opportunist v traplining, territorial v traplining). The four quadrants of each rectangle follow the structure of the 2x2 variance-covariance matrix of rates of evolution in bill size (lower right quadrant) and body size (upper left quadrant) and their evolutionary covariance (upper right and lower left quadrants). Numbers in each quadrant are the percentage overlaps between posterior distributions for that parameter. Where there is a more definite difference between regimes in the posterior distributions for a parameter (i.e. less overlap), this is indicated by a darker colour. (1) original analyses; (2) the outlier species *Hylonympha macrocerca* pruned from the Jetz phylogeny; (3) both phylogenies pruned to have only species that are present in both datasets; (4) the outlier *Hylonympha macrocerca* as well as all species of the bee hummingbird clade pruned from both phylogenies.

Fig S2. Results of ratematrix sensitivity analyses with two foraging categories, traplining and non-traplining. In the left column are analyses based on the McGuire et al. (2014) phylogeny and in the right column are results based on the Jetz et al. (2012) phylogeny. Each rectangle represents a pairwise comparison between the posterior distributions of evolutionary variance-covariance matrices under different ecological regimes. The four quadrants of each rectangle follow the structure of the 2x2 variance-covariance matrix of rates of evolution in bill size (lower right quadrant) and body size (upper left quadrant) and their evolutionary covariance (upper right and lower left quadrants). Numbers in each quadrant are the percentage overlaps between posterior distributions for that parameter. Where there is a more definite difference between regimes in the posterior distributions for a parameter (i.e. less overlap), this is indicated by a darker colour. (1) original analyses; (2) both phylogenies pruned to have only species that are present in both datasets; (3) the outlier *Hylonympha macrocerca* as well as all species of the bee hummingbird clade pruned from both phylogenies.

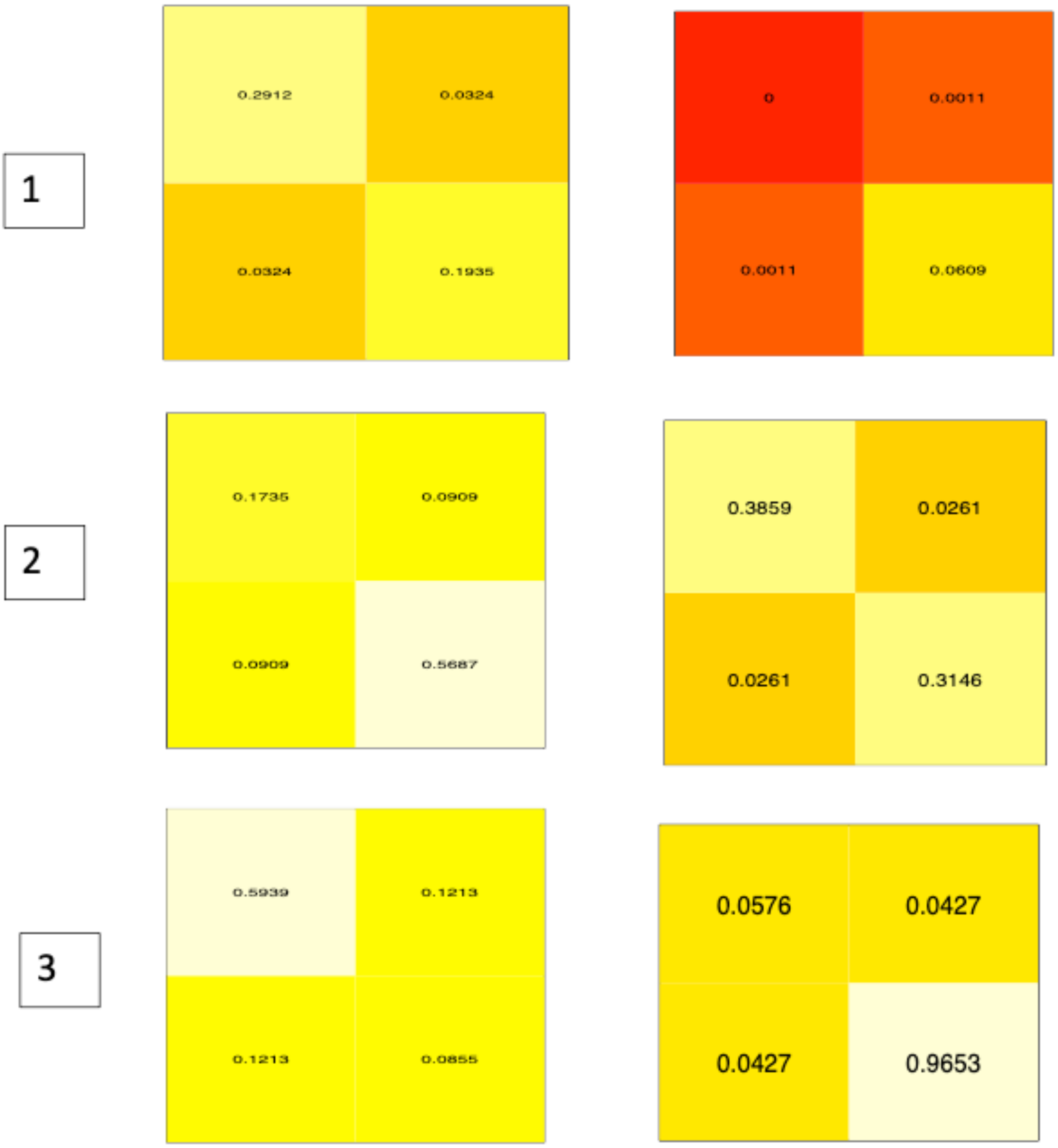
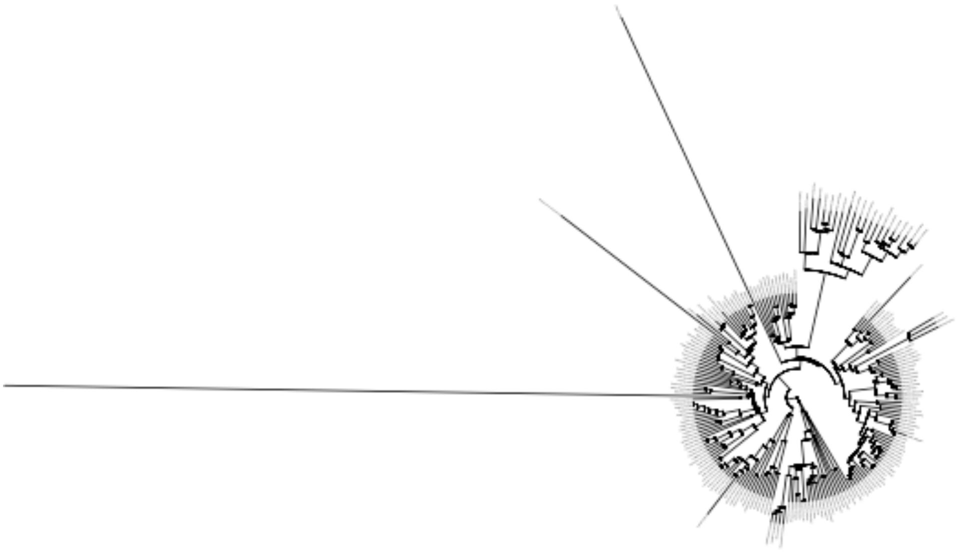


Fig S3. Bayestraits variable rates analyses of correlated trait evolution. Branch length is proportional to the overall rate of evolution. MCMC chains were run in duplicate for 110 million generations with a burnin of 10 million generations for both the Jetz et al. (2012) and the McGuire et al. (2014) phylogenies. The long branches are ones which were pruned in sensitivity analyses.

McGuire



Jetz

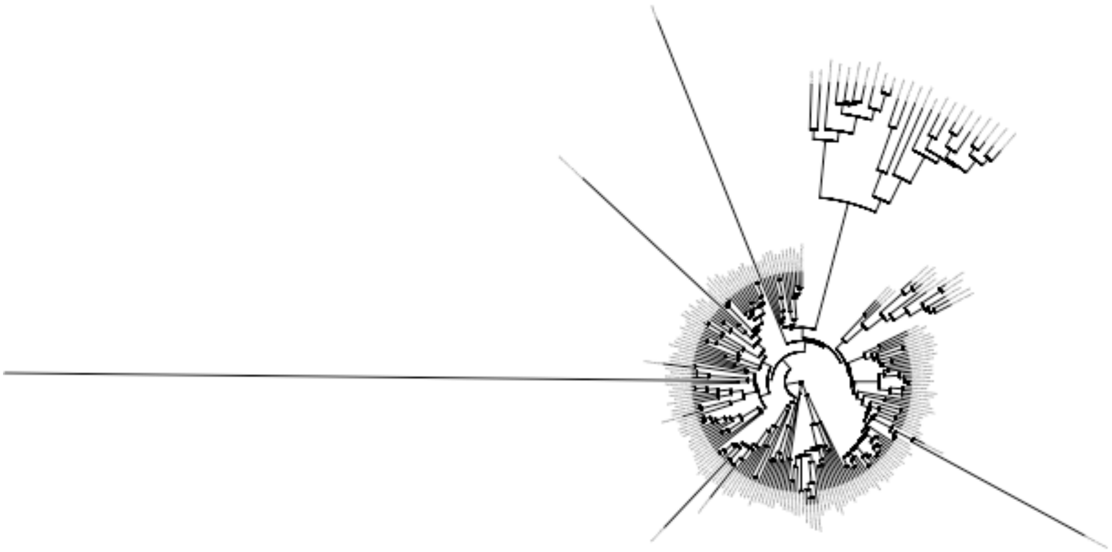


Fig S4. Analysis of state-dependent correlated trait evolution, accounting for background rate heterogeneity with musscrat. The posterior distributions for the relative overall rate of bill and body size evolution are compared for different ecological regimes: traplining in red, opportunism in yellow, and territorialism in blue.

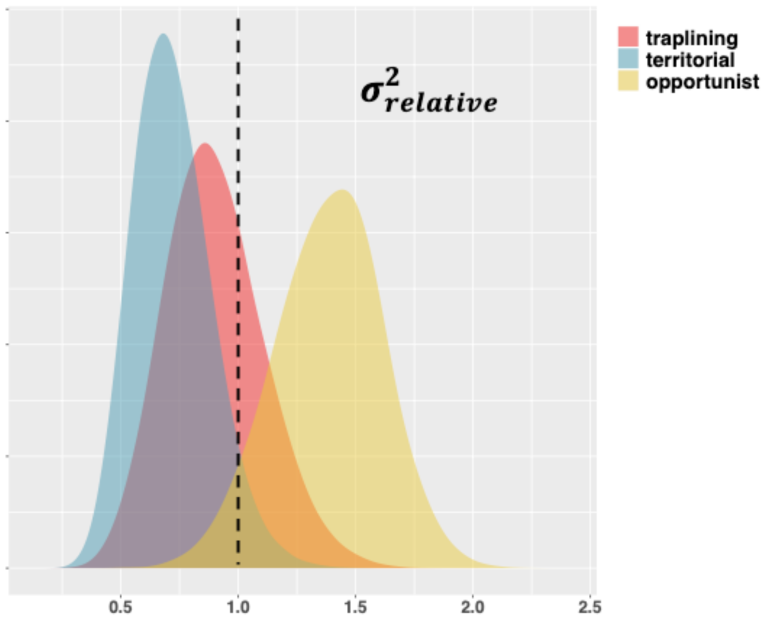


Fig. S5 Phylogeny and transition rates on the McGuire et al. (2014) phylogeny

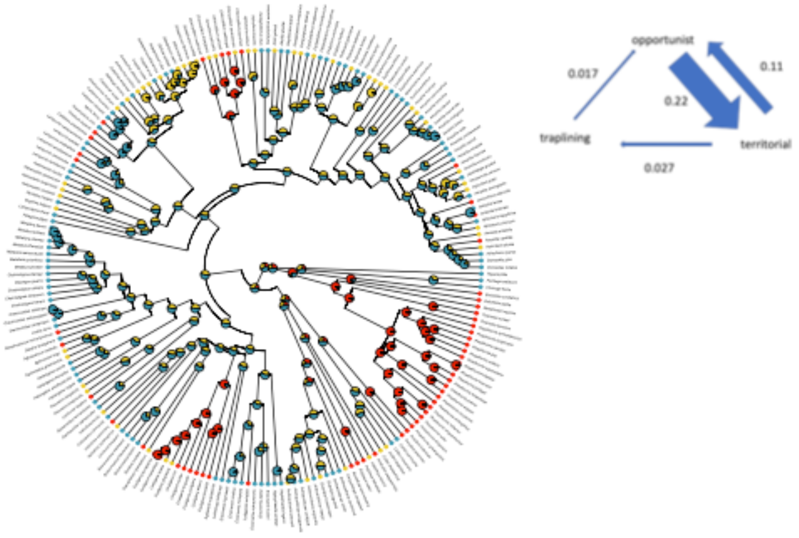


Table S6. FitDiscrete model comparison for analyses based on the Jetz et al. (2012) tree (a) and the McGuire et al. (2014) tree (b).

a)

Model	parameters	likelihood	AIC
All Rates Different	6	-215.79	443.59
Constrained Transition Rates	4	-215.79	439.59

b)

Model	parameters	likelihood	AIC
All Rates Different	6	-187.94	387.87
Constrained Transition Rates	4	-187.94	383.87

Table S7. SecSSE model comparison with Jetz et al. (2012) phylogeny, with one, four, or six separate transition rates. CR= constant rates; CTD= concealed trait-dependent; ETD= examined trait-dependent; sp= speciation; ext= extinction

model	likelihood	parameters	AIC	delta AIC
CTD_sp_4	-929	8	1874	0
CTD_sp_6	-929	10	1878	4
CTD_sp_1	-945	5	1900	26
ETD_sp_6	-945	10	1910	36
ETD_sp_4	-950	8	1916	42
CTD_ext_4	-950	8	1916	42
CR_4	-954	6	1920	46
CTD_ext_6	-950	10	1920	46
ETD_ext_4	-953	8	1922	48
CR_6	-954	8	1924	50
ETD_sp_1	-957	5	1924	50
ETD_ext_6	-953	10	1926	52
ETD_ext_1	-963	5	1936	62
CR_1	-966	3	1938	64
CTD_ext_1	-965	5	1940	66

Table S8. SecSSE model comparison with McGuire et al. (2014) phylogeny, with one, four, or six separate transition rates. CR= constant rates; CTD= concealed trait-dependent; ETD= examined trait-dependent; sp= speciation; ext= extinction

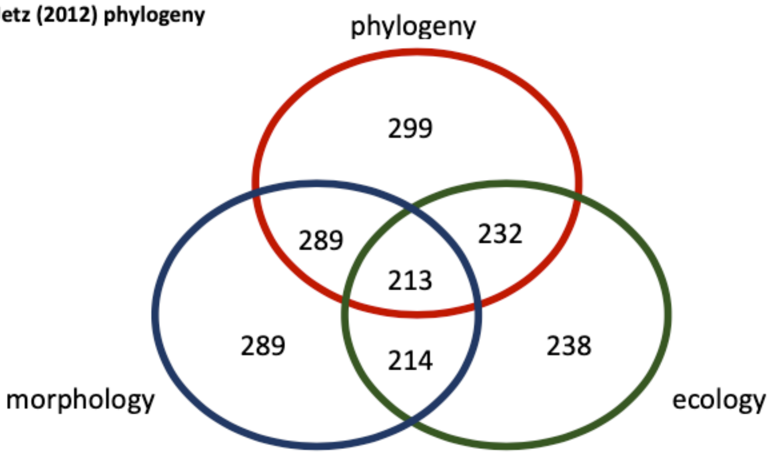
model	likelihood	parameters	AIC	delta AIC
CTD_sp_4	-1082	8	2180	0
CTD_sp_6	-1082	10	2184	4
ETD_sp_4	-1094	8	2204	24
ETD_sp_6	-1092	10	2204	24
CTD_sp_1	-1103	5	2216	36
CR_4	-1103	6	2218	38
ETD_ext_4	-1101	8	2218	38
CTD_ext_4	-1102	8	2220	40
CR_6	-1103	8	2222	42
ETD_ext_6	-1101	10	2222	42
ETD_sp_1	-1117	5	2244	64
CR_1	-1121	3	2248	68
ETD_ext_1	-1120	5	2250	70
CTD_ext_1	-1121	5	2252	72

Table S9. SecSSE model comparison with two foraging classes, trapliners and non-trapliners. CR= constant rates; CTD= concealed trait-dependent; ETD= examined trait-dependent; sp= speciation; ext= extinction

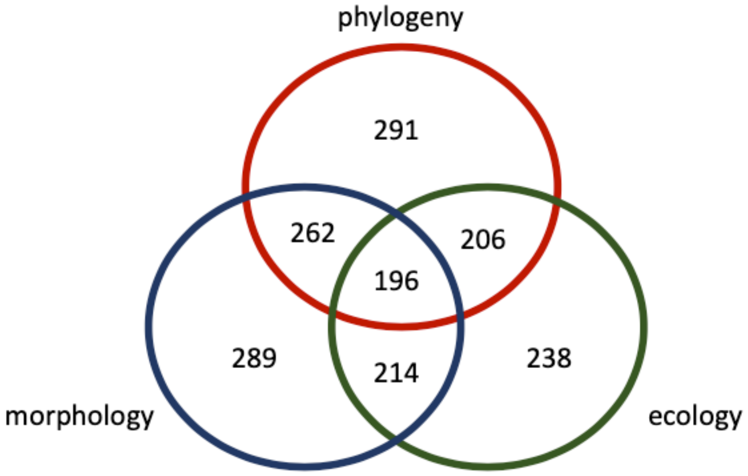
model	likelihood	parameters	AIC	delta AIC
CTD_sp_2	-827	5	1664	0
ETD_sp_2	-847	5	1704	40
CTD_ext_2	-849	5	1708	44
CR_2	-851	4	1710	46
ETD_ext_2	-851	5	1712	48

Fig. S10 Number of species for which data are available on phylogeny, morphology and ecology.

Jetz (2012) phylogeny



McGuire (2014) phylogeny



S11 List of species not shared by the Jetz et al. (2012) and McGuire et al. (2014) phylogenies

Lepidopyga lilliae
Sternoclyta cyanopectus
Hylonympha macrocerca
Amazilia luciae
Amazilia cyanifrons
Thalurania glaucopis
Campylopterus curvipennis
Chlorestes notata
Chlorostilbon russatus
Chlorostilbon gibsoni
Chlorostilbon forficatus
Hylocharis humboldtii
Chaetocercus jourdanii
Chaetocercus heliodor
Mellisuga helenae
Heliomaster constantia
Oxypogon guerinii
Oreotrochilus adela
Ramphomicron dorsale
Aglaiocercus berlepschi
Phlogophilus harteri
Lophornis helenae
Lophornis brachylophus
Lophornis ornatus
Discosura langsdorffi
Discosura longicaudus
Boissonneaua mathewsii
Eriocnemis vestita
Eriocnemis isabellae
Loddigesia mirabilis
Augastes lumachella
Calothorax pulcher
Heliothryx auritus
Amazilia boucardi
Amazilia beryllina
Amazilia saucerottei
Leucippus taczanowskii
Thalurania watertonii
Campylopterus phainopeplus
Chlorostilbon olivaresi
Chlorostilbon lucidus
Chlorostilbon auriceps
Chlorostilbon alice
Chlorostilbon stenurus
Chaetocercus berlepschi
Chaetocercus astreans
Doricha enicura
Atthis ellioti
Metallura iracunda
Chalcostigma heteropogon
Oreotrochilus leucopleurus
Aglaiocercus kingi
Heliangelus mavors
Lophornis stictolophus
Lophornis adorabilis
Lophornis magnificus
Lophornis gouldii
Discosura letitiae
Urosticte benjamini
Eriocnemis mirabilis
Eriocnemis godini
Haplophaedia assimilis
Coeligena lutetiae
Augastes scutatus
Heliactin bilophus

Chapter 6

General Discussion

In this thesis I have explored evolution spanning micro-, macro- and mega- evolutionary timescales and I have sought to bridge the gap in our understanding of how evolution translates from shorter to longer timescales. Here are my conclusions to the questions posed in the introduction to this thesis.

Chapter 2: How do the tempo and mode of evolution translate from microevolutionary to macroevolutionary timescales? Are there common underlying processes at play over all timescales or are there special processes that need to be invoked to account for macroevolutionary patterns? Hence, is macroevolution predictable on the basis of microevolution?

In chapter 2 I investigated two rival hypotheses for a common pattern in evolution, relative conservatism on the microevolutionary level interrupted by macroevolutionary bursts. One hypothesis holds that these bursts are triggered by shifts in adaptive peaks themselves, driven by evolutionary events such as the colonisation of islands, the evolution of 'key innovations', or relatively abrupt environmental change. These fluctuations would then be disconnected from the continuum of typical fluctuations occurring over microevolutionary timescales. We must consider the alternative hypothesis that this pattern is simply an emergent consequence of lineages traversing a rugged adaptive landscape and that there is no discontinuity between micro- and macro- evolutionary processes: the evolutionary continuum hypothesis. Certain processes can lead to emergent patterns that are not immediately predictable from the patterns they produce in the short term. Indeed, I have shown that a model inspired by the evolutionary continuum hypothesis fits the observed pattern of divergence in animal body sizes over time equally well as a model with discrete shifts.

Chapter 3: What is the relationship between macroevolutionary rates of speciation and the rates of component steps of the speciation cycle, encompassing the evolution of geographical, reproductive and ecological isolation between incipiently diverging species?

In chapter 3 I examined the connection between macroevolutionary rates of speciation and the rates of steps involved in the speciation cycle happening continuously over shorter timescales. Speciation is not an instantaneous event, but a protracted process. One may expect that macroevolutionary rates of speciation should be positively correlated with proxies for the rates of evolution of geographical, reproductive and ecological isolation between populations. Surprisingly, such correlations have not been found in recent comparative studies. I have looked at a general explanation for this. I analysed the dynamics of mathematical models of 'protracted speciation' with multiple steps. I found that these models predict nonlinearity in the relationship between the macroevolutionary rate of speciation and the rates of component steps of the speciation cycle, as well as interaction effects between the rates of steps. This suggests that there may be key 'rate-limiting' steps involved in speciation and that the rates of other steps may not contribute as much to the rate of the speciation cycle.

Chapter 4: What evolutionary patterns emerge across macro- to mega-evolutionary timescales?

In chapter 3 I examined tempo and mode in the evolution of the allometric scaling relationship between beak size and body size across the adaptive radiation of all living birds. I found that these relationships are conserved within clades over millions of years relative to the variation that exists across birds as a whole, but shifts in the slope and intercept of this relationship have happened many times throughout history. Shifts have accumulated steadily throughout time with no evidence of an early burst or recent acceleration relative to the number of branches in the bird phylogeny. These megaevolutionary shifts are similar to the macroevolutionary shifts modelled in chapter 2. Perhaps these shifts could be explained by analogous processes as well. One possibility is that phenotypic shifts are explained by shifts in adaptive zones triggered by 'key innovations' or an absence of competitors. An interesting alternative is that no such adaptive peak shifts need to be invoked if phenotypic shifts reflect rare traversals of regions in the adaptive landscape with a low density of adaptive peaks.

Chapter 5: Have different rates of speciation associated with different foraging ecologies shaped hummingbird evolution?

In chapter 5 I studied the macroevolution of hummingbirds in relation to foraging ecology. I tested the hypothesis that a traplining ecology is an evolutionary dead end. While transitions to a traplining ecology from territorial hummingbirds seems largely irreversible, this is not statistically associated with reduced rates of diversification. There is thus no evidence of species selection in this case. Perhaps diversification histories are more idiosyncratic in hummingbirds due to more complex details of foraging ecology not considered here. It would be interesting to test the prediction of traplining being an evolutionary dead end in other groups of pollinators.

Implications for Future Work

Often in macroevolutionary models there is a many-to-one mapping between potential processes and the patterns they produce. To distinguish between candidate hypotheses additional information is needed. The evolutionary continuum hypothesis examined in Chapter 2 predicts that micro- evolutionary dynamics should be correlated with rates of macroevolution, while no such correlation is necessarily expected from the alternative adaptive peak shift hypothesis. Specifically, weaker stabilising selection or the presence of disruptive selection and more intraspecific variation could be candidate predictors for faster macroevolutionary rates of phenotypic evolution. This could be tested in future work. Another avenue that could be explored is using the simulation framework developed here to study how rates of macroevolutionary bursts depend on fluctuations in the parameters of the model, such as those that might arise from population bottlenecks. With the application of mathematical modelling, it is possible for original predictions to emerge. The model constructed in Chapter 3 predicts nonlinearity and interaction effects in the relationship between macroevolutionary rates of speciation and the rates of component steps. Multipredictor models of speciation rate could look for evidence of these prediction in the future. In summary, this thesis finds that the tempo and mode of evolution are heterogeneous over different timescales, but our understanding of evolutionary patterns over different timescales can be bridged by understanding how underlying processes interact and scale over time.