Density-dependent diversification in North American wood warblers

Daniel L. Rabosky[1,2,*] and Irby J. Lovette[1,2]

[1]*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA*
[2]*Cornell Laboratory of Ornithology, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA*

Evidence from both molecular phylogenies and the fossil record suggests that rates of species diversification often decline through time during evolutionary radiations. One proposed explanation for this pattern is ecological opportunity, whereby an initial abundance of resources and lack of potential competitors facilitate rapid diversification. This model predicts density-dependent declines in diversification rates, but has not been formally tested in any species-level radiation. Here we develop a new conceptual framework that distinguishes density dependence from alternative processes that also produce temporally declining diversification, and we demonstrate this approach using a new phylogeny of North American *Dendroica* wood warblers. We show that explosive lineage accumulation early in the history of this avian radiation is best explained by a density-dependent diversification process. Our results suggest that the tempo of wood warbler diversification was mediated by ecological interactions among species and that lineage and ecological diversification in this group are coupled, as predicted under the ecological opportunity model.

Keywords: speciation; adaptive radiation; diversification; ecological opportunity; community assembly; species interactions

1. INTRODUCTION

One of the most striking features of evolutionary radiations is a tendency for species-level diversification rates to decline through time. This pattern has long been recognized in the fossil record, where explosive but transient bursts of diversification appear to follow both mass extinctions (Sepkoski 1998) and the invasion of previously unoccupied adaptive zones (Simpson 1953; Stanley 1973). A large number of studies have used molecular phylogenies of extant taxa to document a pattern of early rapid diversification, followed by temporal declines in diversification rates (e.g. Lovette & Bermingham 1999; Harmon *et al.* 2003; Rüber & Zardoya 2005; Weir 2006; Phillimore & Price 2008; Rawlings *et al.* 2008). Although several potential biases can generate spurious shifts in diversification rates inferred from molecular phylogenies (Nee 2001; Revell *et al.* 2005), methodological improvements (Pybus & Harvey 2000; Rabosky 2006*a*) continue to support the phenomenon of declining diversification rates through time in species-level radiations.

One potential biological explanation for this pervasive pattern is that evolutionary radiations are facilitated by ecological opportunity (Schluter 2000), whereby speciation is most likely when resources are abundant and potential competitors are scarce. As a radiation progresses, ecological 'niche space' becomes increasingly saturated, resulting in fewer opportunities for speciation (Walker & Valentine 1984; Valentine 1985). Under such a model, speciation rates are predicted to show density dependence

(Nee *et al.* 1992), because the rise in species diversity through time would be mirrored by a corresponding decline in the speciation rate. In a meta-analysis of 45 avian radiations, Phillimore & Price (2008) found widespread evidence for temporal slowdowns in diversification rates and speculated that ecological constraints on clade growth resulted in density-dependent speciation. Weir (2006) reported that neotropical avian clades with greater numbers of sympatric species showed more pronounced declines in diversification rates through time, which suggests that clade diversity within a particular ecological or biogeographic theatre might itself limit the rate of clade growth. Although these studies are consistent with density-dependent diversification, there have been no formal tests of the process in any evolutionary radiation, because methods have not been available that can discriminate between density dependence and other processes that might also result in temporal declines in diversification rates.

Here, we develop a novel conceptual framework for testing whether diversification rates show density dependence, and we explore the role of this process during the radiation of continental North American wood warblers (Parulidae) in the speciose genus *Dendroica*. *Dendroica* warblers are an ecologically appropriate group in which to test for density dependence diversification, as this process is most likely to be driven by interspecific competitive interactions. *Dendroica* species diversity is high in many local North American assemblages, but the composition of those assemblages is variable among sites (Lovette & Hochachka 2006). The matrix of potential species interactions is even more complex when integrated across the history of this group, as most *Dendroica* species have persisted through climate cycles that would have further scrambled their geographical ranges and spatial

associations. These warblers are a classic example of behavioural niche differentiation, in which co-occurring species differ in subtle aspects of their foraging and breeding behaviour (MacArthur 1958; Price *et al.* 2000). Our previous studies based on a time-calibrated mitochondrial DNA (mtDNA) phylogeny found that the *Dendroica* group underwent an explosive burst of diversification early in its history, followed by a pronounced decline in the rate of lineage accumulation (Lovette & Bermingham 1999). Taken together, these observations suggest the possibility that broad-scale patterns of diversification in the *Dendroica* warblers might be related to ecological interactions among species across evolutionary time-scales.

Our statistical approach extends the birth–death model that has been used previously for inference on diversification rates (Nee *et al.* 1994; Barraclough & Vogler 2002; Nee 2006; Rabosky 2006*b*) to speciation rates that vary continuously through time. We apply the method to an improved phylogeny of *Dendroica* warblers that is complete at the species level and which is based on both mtDNA and nuclear sequence loci. Our results indicate that the observed pattern of speciation in *Dendroica* is best approximated by a density-dependent diversification process and suggest that ecological interactions among species can leave an imprint on evolutionary history that can be reconstructed from molecular phylogenies alone.

2. MATERIAL AND METHODS

(a) *Taxon sampling and phylogenetic analyses*
We reconstructed relationships among all species in the *Dendroica* radiation, including four species traditionally assigned to the genera *Parula*, *Setophaga* and *Wilsonia* that fall within this well-supported clade (Lovette & Bermingham 2002). We sequenced a total of six mitochondrial protein-coding genes and six nuclear intron loci. All genes and loci were obtained from all taxa, except for *Dendroica chrysoparia*, for which we had only older museum skin material, and hence from which we obtained only the mitochondrial-encoded ND2 sequence. Reconstructions were based on a total of 5261 nucleotides of protein-coding mtDNA sequence and 4296 aligned nucleotides of nuclear intron sequence (GenBank accession numbers, specimen data and DNA amplification/sequencing primers are given in tables S1–S3 in the electronic supplementary material). Although the phylogenetic reconstructions included all taxa within the *Dendroica* radiation, our subsequent tests of diversification rates and their potential density dependence employed a pruned tree that excluded lineages restricted to West Indian islands, as those isolated island taxa are very unlikely to have been involved in interspecific interactions in continental warbler communities.

We used BEAST v. 1.4.6 to simultaneously infer topologies and relative divergence times under a relaxed-clock model of sequence evolution (Drummond *et al.* 2006; Drummond & Rambaut 2007). Because the diversification rate analyses described below require only that trees be calibrated to relative time-scales, we used a model of uncorrelated but lognormal distributed substitution rates and fixed the mean rate at 1.0. We recognized four partitions with independent evolutionary parameters (three mitochondrial codon positions plus nuclear DNA) and assigned a GTR+G+I model of sequence evolution to each, following analyses with MrModeltest v. 2.2 (Nylander 2004). We performed 11 runs of Markov

chain Monte Carlo (MCMC) on the combined dataset, sampling parameters every 30 000 generations and discarding the first five million generations from each as burn-in. Post-burn-in parameters and trees were combined across runs for a total of 341 million generations of MCMC sampling. We placed uniform [0, 100] priors on parameters of the substitution rate matrix and default priors on all other parameters. We assessed convergence on the posterior distribution by calculating effective sample sizes for evolutionary parameters using Tracer v. 1.4 (Drummond *et al.* 2006).

(b) *Models and parameter estimation*
To test for density-dependent diversification, one cannot simply compare the likelihood of phylogenetic data under a density-dependent diversification model to the corresponding likelihood under a constant-rate model of diversification. There are many reasons why diversification rates might appear to decline gradually through time that have nothing to do with density-dependent cladogenesis. For example, both incomplete taxon sampling and artefacts of phylogeny reconstruction can result in spurious declines in diversification rates through time, even when rates have not changed (Nee *et al.* 1994; Nee 2001; Revell *et al.* 2005). It may be the case that density-dependent models fit such data better than constant-rate models, simply because these models provide a crude approximation of continuous declines in diversification rates. In the electronic supplementary material (figure S1), we show that a constant-rate diversification process in conjunction with incomplete taxon sampling can strongly mimic density-dependent diversification when the data are simply fitted with density-dependent and constant-rate diversification models. We argue that the relevant null hypothesis for density-dependent diversification is a model where diversification rates are permitted to vary continuously through time but not directly as a function of the number of lineages in existence.

We considered both exponential and linear models of density-dependent diversification (Nee *et al.* 1992; Rabosky 2006*a*). Under the exponential model, the speciation rate λ is modelled as

$$\lambda(t) = \lambda_0 N_t^{-x}, \tag{2.1}$$

where λ_0 is the initial speciation rate; N_t is the number of lineages in existence at time t in a reconstructed phylogeny; and x determines the magnitude of the rate change as a function of N_t. Note that $x=0$ implies constant speciation through time. For the linear model,

$$\lambda(t) = \lambda_0 \left(1 - \frac{N_t}{K}\right), \tag{2.2}$$

where K is analogous to the carrying capacity parameter of population biology. Note that this model is commonly known as the logistic model in population biology, but nonetheless specifies a linear decline in the speciation rate. In the palaeontological literature, several studies have addressed density-dependent clade growth using logistic models (e.g. Walker & Valentine 1984). However, if the rate of decline in the speciation rate itself declines as the number of species rises, then the exponential model will fit the data better.

We have previously used theory based on the birth–death process (Nee *et al.* 1994) to fit these models to phylogenetic data (Rabosky 2006*a*; Rawlings *et al.* 2008). As a null hypothesis for density-dependent diversification, we considered a simple model where the speciation rate varies continuously

through time but is independent of the number of lineages in existence at any point in time. We modelled speciation as a linear function of time

$$\lambda(t) = \lambda_0 \left(1 - \frac{t}{K}\right). \tag{2.3}$$

In this model, which we refer to as the continuous-decline model, the rate of change of the speciation rate is independent of time (e.g. there is no second derivative), and the magnitude of the rate decline increases as $t \to K$. Although many approaches could be used to model time-dependent speciation rates, we chose this simple model because we felt that it provided a reasonable approximation of monotonic changes in environmental variables through time that might influence diversification rates.

We do not treat diversification under the birth–death process with non-zero extinction for several reasons. First, we have previously shown that patterns of early rapid diversification as inferred from molecular phylogenies of extant taxa can only be explained by declining speciation rates through time and not by increasing extinction rates (Rabosky & Lovette in press). Second, high but constant extinction rates will erase the signature of such 'explosive-early' diversification from molecular phylogenies, rendering it impossible to observe even dramatic declines in speciation rates through time (Rabosky & Lovette in press). For these reasons, extinction rates estimated from phylogenies that appear to undergo temporal declines in diversification rarely differ from zero (Weir 2006; Rabosky & Lovette in press).

To find the likelihood of phylogenetic data under the continuous-decline model, we used the general probability model developed by Nee *et al.* (1994): this approach was used to model time-varying speciation and extinction rates in Rabosky & Lovette (in press). Consider a general birth process, where existing lineages give birth to new lineages at a per lineage, time-varying speciation rate $\lambda(t)$. Let t_i represent the birth time of each of the N lineages in the phylogeny which survive to the present (time T). The likelihood of the phylogenetic data is given by

$$L = (N-1)! \prod_{i=3}^{N}\{\lambda(t_i)\} \prod_{i=3}^{N}\{\xi_i\}\{\xi_2^2\}, \tag{2.4}$$

where

$$\xi_i = \exp\left[\int_{t_i}^{T} -\lambda(s)\mathrm{d}s\right] \tag{2.5}$$

with t_2 corresponding to the time of the initial bifurcation in the tree. Equation (2.4) is identical to Nee *et al.* (1994, eqn (20)) with no extinction term. This expression considers only $N-2$ speciation events, because the first two speciation events must have occurred; if they had not, no phylogenetic tree would exist to be observed (Nee *et al.* 1994). The ξ_2 terms in equation (2.4) corresponds to these basal branches, and there are two of them.

Equations for modelling time-varying speciation rates were obtained for the continuous-decline model by deriving the appropriate analytical expression for equation (2.3) in conjunction with equations (2.4) and (2.5). Models were fitted to phylogenetic data using Nelder–Mead and Broyden–Fletcher–Goldfarb–Shanno algorithms as implemented in the 'optim' routine for the R programming language. All optimizations were repeated 20 times with random starting parameters to decrease the possibility that solutions reflect local maxima.

(c) *Diversification analyses*

If ecological opportunity or niche availability facilitated speciation during the radiation of *Dendroica* warblers, we predicted that (i) diversification rates would decline significantly through time and (ii) models specifying density dependence of speciation rates would fit the observed data better than a model where rates decline continuously through time.

We first tested whether previous conclusions about declining diversification rates in the group (Lovette & Bermingham 1999) are robust to the additional data, taxon sampling and analytical methodologies presented in this paper. We computed the γ statistic (Pybus & Harvey 2000) for ultrametric trees recovered with the BEAST analysis, where $\gamma < 0$ implies decelerating diversification through time. It is well known that incomplete taxon sampling can result in a perceived temporal decline in diversification rates (Nee *et al.* 1994). Although we included all nominate members of the continental *Dendroica* radiation in our analysis, it is possible that undescribed or morphologically cryptic species could have resulted in a spurious decline in diversification rates over time. To explore the effects of missing species on our analysis, we determined the number of missing lineages that would render the observed γ-statistic insignificant. We assumed that our sample of $n = 25$ lineages represented a proportion f of the true number of lineages and simulated sets of 5000 phylogenies under a pure-birth model of cladogenesis for values of f from 0.25 to 1.0. We calculated the γ-statistic for all simulated trees and determined the 0.05 percentile of the distribution of γ for each f; this value corresponds to the lower bound of the 95% CI around the null hypothesis that γ is not significantly less than zero.

We then tested whether the tempo of lineage accumulation during the *Dendroica* radiation is best approximated by density-dependent or continuous-decline models of diversification. We compared the likelihood of the warbler phylogeny under these competing classes of models using the Akaike information criterion (AIC). We had no *a priori* predictions as to whether density-dependent diversification should follow an exponential or linear model. We therefore computed the following test statistic:

$$\Delta\mathrm{AIC_{TS}} = \mathrm{AIC_{H0}} - \mathrm{AIC_{H1}}, \tag{2.6}$$

where $\mathrm{AIC_{H0}}$ is the AIC score of the null hypothesis model (continuous-decline) and $\mathrm{AIC_{H1}}$ is the AIC score corresponding to the best-fit hypothesis model (density-dependent exponential or linear). Thus, a positive $\Delta\mathrm{AIC_{TS}}$ implies that density-dependent models fit the data better than the continuous-decline model. We computed $\Delta\mathrm{AIC_{TS}}$ for the maximum clade credibility (MCC) tree, which is an estimate of the tree with the maximum *a posteriori* probability. The MCC tree is the tree for which the product of posterior probabilities across all nodes present in the tree is greater than for any other trees in the posterior distribution. To avoid conditioning our results on any particular topology and branch lengths, we computed the distribution of $\Delta\mathrm{AIC_{TS}}$ over the posterior distribution of trees sampled using MCMC, with the prediction that the continuous-decline model would consistently provide a poorer fit to the data than the density-dependent models.

The analyses described above are critically dependent on the assumption that density-dependent models will not overfit the data in the absence of density-dependent diversification. To test this assumption, we investigated
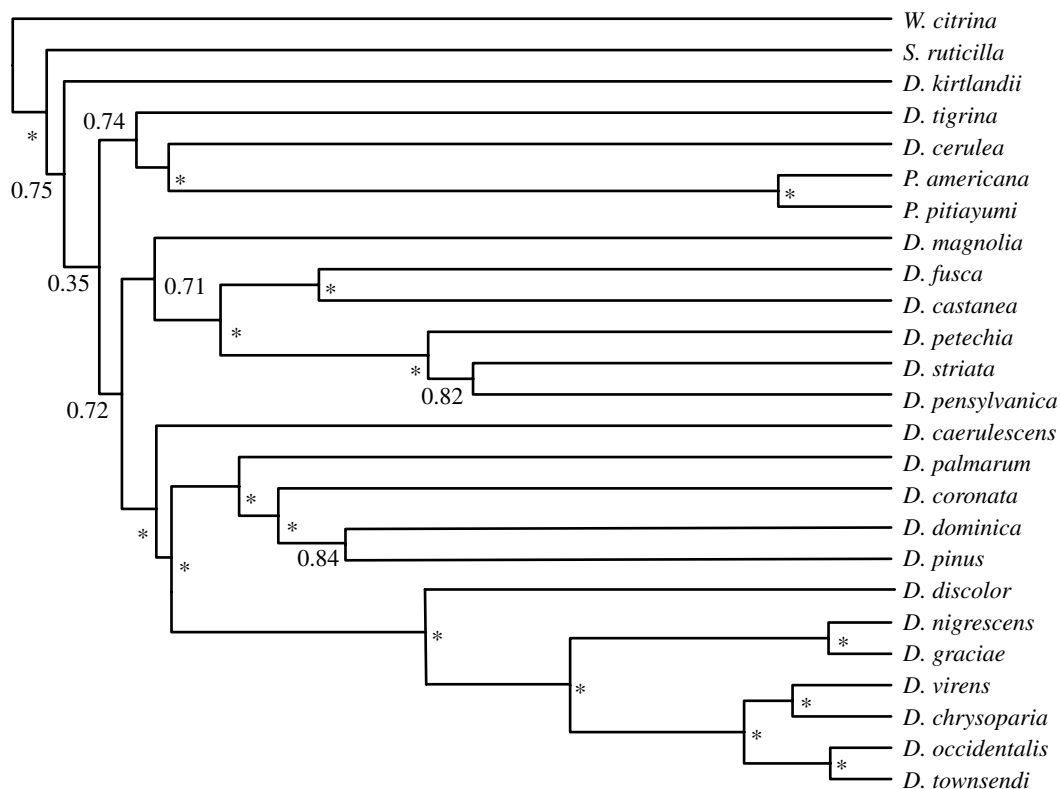
Figure 1. Maximum clade credibility (MCC) tree from Bayesian analysis of all continental North American *Dendroica* wood warbler species. Nodes marked with asterisks are supported by posterior probabilities of more than 0.95. Tree is based on more than 9 kb of mtDNA and nuclear intron sequence. Branch lengths are proportional to absolute time.

type I error rates for constant-rate phylogenies simulated under both pure birth and continuous-decline models of diversification, assuming both complete and incomplete taxon sampling. For the pure birth model, we simulated 1000 trees of $N=25$ taxa under a constant speciation process and tabulated the distribution of $\Delta\mathrm{AIC_{TS}}$. To further control for the possibility that incomplete taxon sampling could result in high type I error rates, we tabulated the distribution of the test statistic for constant-rate phylogenies simulated with different levels of incomplete sampling (f), as described above for the γ-statistic analyses.

We used the method described in Rabosky & Lovette (in press) to simulate phylogenetic trees under the continuous-decline model of diversification (electronic supplementary material, supplementary methods). This approach enables the continuous-time simulation of phylogenetic trees with time-varying rate parameters. We simulated clade growth under the continuous-decline model assuming both 5-fold and 10-fold reductions in the speciation rate through time. We found parameters of the continuous-decline model (λ_0, K) which would result in an expected value of $N=25$ lineages after $t=1.0$ time steps, where the expected number of lineages is calculated as

$$n(t) = 2 \exp\left(\int_0^{t=1} \lambda(s)\mathrm{d}s\right), \qquad (2.7)$$

after Nee *et al.* (1994). We further required that model parameters satisfy the relationships $\lambda_0=5\lambda_1$ and $10\lambda_1$ for 5-fold and 10-fold declines, respectively. To simulate incomplete sampling under the continuous-decline model, we found parameters corresponding to 5-fold and 10-fold declines, which were expected to result in 33 ($f=0.75$), 50 ($f=0.5$) or 100 ($f=0.25$) lineages at the end of the

simulation. Simulated trees were then randomly pruned to the desired sampling level. All phylogenetic simulations were conducted using a modified version of the birth–death tree simulation algorithm from the GEIGER package for R (Harmon *et al.* 2008).

3. RESULTS

Phylogenetic trees generated under a relaxed-clock model of sequence evolution (figure 1) strongly supported previous findings that diversification rates in North American wood warblers have declined through time. A lineage-through-time (LTT) plot clearly indicates an excess of lineages early in the history of the wood warbler radiation (figure 2a) relative to the expected rate of lineage accumulation under a constant-rate model of diversification. Calculated γ-statistics for the MCC tree (-3.48) and for the posterior distribution of topologies and branch lengths (2.5 and 97.5% quantiles of -3.63 and -3.19, respectively) indicate highly significant temporal declines in diversification rates ($p<0.001$). This result is robust to assumptions about missing taxa: γ for the MCC tree is significant even when we assume that our tree contains only 25% of North American *Dendroica* species ($p=0.019$). There is little overlap between the distribution of γ calculated from the posterior distribution of phylogenetic trees and the corresponding null distributions assuming complete and incomplete sampling (figure 2b).

Model-based analyses of diversification provided strong support for density-dependent diversification in *Dendroica* wood warblers (table 1). Among the candidate models, the density-dependent exponential model provided the best approximation to the observed pattern of lineage accumulation through time ($\Delta\mathrm{AIC_{TS}}=10.27$).

Figure 3. Maximum-likelihood reconstruction of speciation-through-time curve under overall best-fit model (density-dependent exponential). Rates are given in lineages per time unit assuming that the basal divergence occurred 1.0 time units before the present.
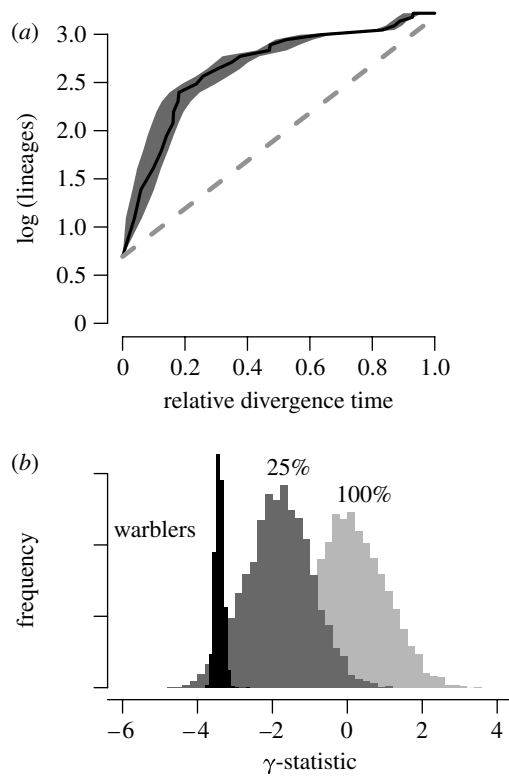
Figure 2. (a) Log-lineage through time (LTT) plot for North American wood warblers. Black line indicates LTT curve for the MCC tree (figure 1), and grey shading indicates 95% quantiles on the number of lineages at any point in time as inferred from the posterior distribution of phylogenetic trees sampled with MCMC. The dashed line indicates expected rate of lineage accumulation under constant-rate diversification with no extinction. Lineages accumulate quickly in the early phases of the radiation relative to the constant-rate diversification model. (b) Posterior distribution of the γ-statistic for wood warblers (black) in comparison with the corresponding null distributions, assuming either complete ($f = 1$) or incomplete ($f = 0.25$) sampling. Negative values of γ relative to the null distribution indicate decelerating diversification through time.

Table 1. Summary of diversification models fitted to the MCC tree (figure 1) for the North American *Dendroica* wood warbler radiation.

model	log likelihood	ΔAIC^a
density-dependent, exponential	50.47	0
density-dependent, linear	48.42	4.09
linear	45.33	10.27
pure birth	40.45	18.03

[a] Difference in AIC scores between each model and the overall best-fit model.

Diversification rates reconstructed using maximum-likelihood parameter estimates for this best-fit model ($\lambda_0 = 71$, $x = 1.47$) suggest an explosive burst of diversification early in the history of the radiation (figure 3), followed by a rapid decline in per lineage diversification rates. In the electronic supplementary material (figures S2 and S3), we provide expected LTT curves for the three fitted rate-variable models and discuss how differences in these patterns relate to our ability to discriminate between density-dependent and continuous-decline models.
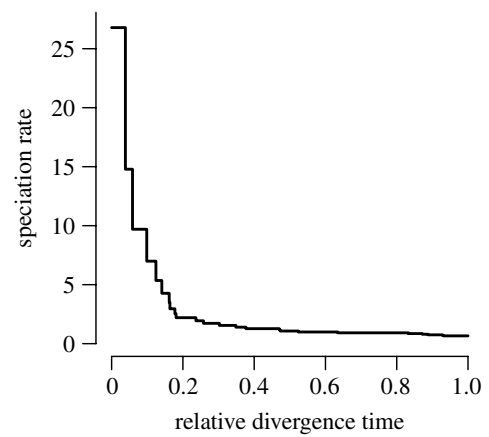
To test whether our results were robust to uncertainty in phylogeny estimation and assumptions about the completeness of taxon sampling, we tabulated the distribution of ΔAIC_{TS} from the posterior distribution of trees and branch lengths sampled using MCMC. We compared this distribution with the null distribution generated by simulating phylogenies under both constant-rate and continuous-decline models of diversification with and without incomplete sampling. If ΔAIC_{TS} is characterized by an exceptionally high type I error rate, we expect density-dependent models to consistently fit the data better than continuous-decline models, even when diversification rates have not declined in density-dependent fashion.

Under both constant-rate and continuous-decline simulations (figure 4), we find that the null distribution of ΔAIC_{TS} is consistently greater than zero. This implies that, on average, density-dependent models fit the data slightly better than the continuous-decline model, even when the data are simulated under a continuous-decline diversification process. However, it is extremely unlikely that our results can be explained by this weak bias in favour of density-dependent models. Although a constant diversification process in conjunction with incomplete taxon sampling can generate the appearance of temporally declining diversification (e.g. figure 2b; null distributions of γ), our results reject the possibility that this artefact underlies the much greater fit of density-dependent models to the wood warbler data (figure 4a). The observed ΔAIC_{TS} statistic for the MCC tree (10.27) indicates a significantly greater fit of density-dependent models relative to the null distribution when sampling is assumed to be complete ($p < 0.001$) or incomplete at 75% ($p < 0.001$), 50% ($p < 0.001$) and 25% ($p = 0.008$) levels. Moreover, we find little evidence that a continuous-decline process in conjunction with incomplete sampling (figure 4b,c) can explain the observed distribution of ΔAIC_{TS} for the wood warblers. The ΔAIC_{TS} test statistic for the MCC tree is significantly greater than expected under the null distribution assuming a 5-fold decline in diversification across all levels of incomplete sampling ($p \leq 0.01$). This result is not significant only when we assume at least 10-fold declines in diversification with 25% sampling ($p = 0.085$).
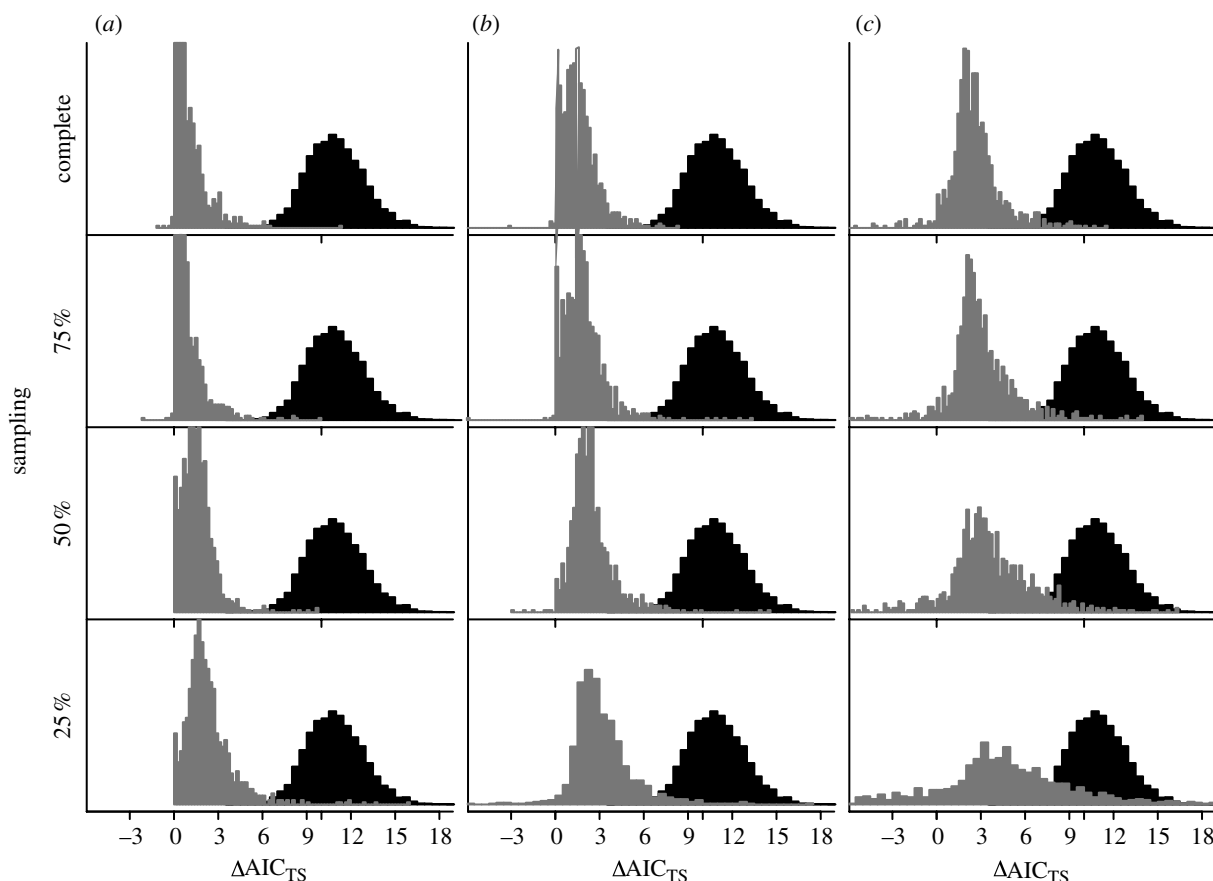
Figure 4. Distribution of ΔAIC_{TS} test statistic as tabulated from the posterior distribution of wood warbler phylogenies sampled using MCMC (black). Larger ΔAIC_{TS} values indicate better fit of density-dependent diversification models relative to continuous-decline models. Null distributions of ΔAIC_{TS} statistic (grey) were tabulated from phylogenies simulated under constant-rate and continuous-decline diversification models. Null distributions in (*a*) correspond to constant-rate phylogenies; distributions in (*b,c*) are phylogenies simulated under 5-fold and 10-fold declines in speciation under the continuous-decline model (equation (2.3)). We further tabulated null distributions under each model assuming taxon sampling was complete or incomplete at 75, 50 and 25% levels. Density-dependent models consistently fit the data better than the continuous-decline model, even when large numbers of missing taxa are assumed. The ΔAIC_{TS} statistic for the MCC tree is significant ($p < 0.05$) under all diversification/sampling scenarios except the 10-fold decline with 25% sampling ($p = 0.085$).

4. DISCUSSION

We present a new conceptual framework for detecting density-dependent diversification rates and report evidence for this process during the radiation of North American wood warblers. Although previous studies have suggested that density dependence might account for the apparent deceleration in the rate of cladogenesis observed in molecular phylogenies (Nee *et al*. 1992; Weir 2006; Phillimore & Price 2008), ours is the first to explicitly test whether patterns of speciation are more consistent with density dependence than with other processes that can result in temporally declining diversification.

Despite the size of our DNA sequence dataset (9557 combined nucleotides), we were unable to resolve phylogenetic relationships among some early diverging lineages (figure 1; nodes with less than 0.95 posterior probability). However, our results are robust to this uncertainty in phylogeny estimation. The narrow confidence limits on the reconstructed number of lineages as a function of time (figure 2*a*) indicate that even alternative phylogenetic relationships among *Dendroica* taxa show a similar pattern of lineage accumulation. Likewise, the posterior distribution of γ suggests that virtually all topologies and branch lengths sampled using MCMC

show this pattern of early and rapid diversification (figure 2*b*). The significantly better fit of density-dependent models to the MCC tree (figure 1; table 1) is robust to uncertainty in topology and branch length estimation as well as assumptions about sampling completeness (figure 4). Although incomplete taxon sampling generates a pattern of lineage accumulation through time that can mimic temporally declining diversification, we find that assuming large numbers of missing taxa does not change our result (figure 4). The ΔAIC_{TS} test statistic shows a bias favouring density dependence over the continuous-decline model (figure 4); however, more than 75 taxa would need to be missing from our analysis for this to pose a problem under all but the steepest declines in diversification. We consider this degree of incompleteness a highly unlikely scenario given that the North American avifauna is well characterized and many *Dendroica* species have been the focus of densely sampled phylogeographic studies.

(a) *Ecological causes of density-dependent speciation*

A relaxation of interspecific competition in conjunction with the availability of diverse resources has been characterized as 'ecological opportunity' (Schluter 2000)

and is widely hypothesized to drive both lineage and phenotype diversification during evolutionary radiations (Simpson 1953; Erwin *et al.* 1987; Foote 1996). This is an intuitively appealing and theoretically plausible model (Walker & Valentine 1984; Gavrilets & Vose 2005) that can explain our finding of density-dependent diversification rates in wood warblers. On a mechanistic level, ecological opportunity can facilitate higher per lineage rates of speciation by increasing the likelihood that a population will split and successfully occupy multiple peaks on the adaptive landscape.

One alternative to density-dependent speciation is that temporal declines in diversification are attributable to increasing extinction rates during evolutionary radiations. Because the net rate of species diversification through time is the difference between the speciation rate and the extinction rate, a density-dependent increase in the extinction rate during evolutionary radiations would also generate density-dependent declines in the net diversification rate. There are theoretical reasons why rates of extinction might increase during evolutionary radiations (e.g. Ricklefs & Cox 1972). For example, a limit on total resource availability implies that increasing species diversity will result in lower mean population sizes per species (Levinton 1979; Hubbell 2000). Because population size is a determinant of extinction probability, extinction rates might increase with the number of species. However, we have previously shown that temporally declining speciation is the only process that can leave a signature of early rapid diversification in a molecular phylogeny of extant taxa (Rabosky & Lovette in press). This result does not rule out the possibility that some evolutionary radiations are characterized by density-dependent extinction rates, but it does suggest that it would be difficult to infer such a process from molecular phylogenies alone.

(b) *Ecological opportunity and continental evolutionary radiations*

It is widely thought that ecological opportunity might underlie the dramatic ecological diversification of many clades on islands (Baldwin & Sanderson 1998; Lovette *et al.* 2002) and in freshwater lakes (Bernatchez *et al.* 1999; Seehausen 2006). It is perhaps unsurprising that colonizing species in these insular environments would encounter a combination of high resource availability and a paucity of competing species, as the formation of these environments results in novel habitats that are characterized, at least initially, by low species richness. However, continental radiations occur against a complex ecological background that differs from comparatively simple island systems, and it is unclear whether the processes and conditions that facilitate adaptive radiations on islands are also important during continental radiations (Barraclough *et al.* 1999). It is possible that conditions of ecological opportunity that might exist during the early stages of island radiations generally do not occur in continental systems.

The few quantitative analyses of continental radiations have yielded mixed results: some radiations show patterns of diversification consistent with a role for ecological opportunity (e.g. Lovette & Bermingham 1999; Harmon *et al.* 2003; Rabosky *et al.* 2007*a,b*), whereas others do not (McPeek & Brown 2000; Turgeon *et al.* 2005). Other radiations show patterns of lineage but not phenotype diversification consistent with ecological opportunity, indicating that these two aspects of diversification need not be coupled (Kozak *et al.* 2006). Still other studies (Irschick *et al.* 1997) suggest that even closely related taxa can experience different patterns of diversification on continents and islands.

(c) *Alternatives to ecological opportunity*

Although ecological opportunity has been a favoured explanation for temporal declines in speciation rates (e.g. Weir 2006; Phillimore & Price 2008), these results may also be consistent with other processes that entail no direct relationship between speciation and ecological opportunity. For example, it is possible that species interactions influence various aspects of geographical range size, which might then influence the probability of allopatric speciation. Theoretical work suggests that species interactions can limit geographical ranges (Case *et al.* 2005), and there is some evidence that geographical range size is positively correlated with diversification rates (Rosenzweig 1995; Cardillo *et al.* 2003; but see Jablonski & Roy 2003). During evolutionary radiations, mean range size might decline as the number of species in a particular biogeographic theatre increases. If declining range size results in lower per lineage speciation rates, then diversification rates as inferred from molecular phylogenies could show density dependence. We note that this model of diversification does not imply that adaptive radiation underlies the temporal declines in speciation commonly observed during evolutionary radiations (e.g. Phillimore & Price 2008), because it allows the possibility that behavioural interference and other interactions unrelated to resource use might drive the pattern.

It is also possible that apparent density dependence of speciation rates could arise as an artefact of phylogeny reconstruction and branch length estimation. It is well known that underparametrized models of sequence evolution can lead to the impression of temporal declines in diversification (Revell *et al.* 2005). Although we reconstructed phylogenies using a complex model of sequence evolution with multiple data partitions, it is not clear whether existing models of molecular evolution are sufficient for reconstructing substitutional histories along the deep internal branches of a phylogenetic tree. Rabosky & Lovette (in press) pointed out that explosive-early radiations pose a paradox: evidence from the fossil record suggests that virtually all groups diversify with appreciable background extinction rates, yet high extinction rates render it impossible to observe such rapid radiations in molecular phylogenies. One possible solution is that the pattern is, at least in part, attributable to inadequacy of molecular evolutionary models in general use, and this topic clearly deserves a much more comprehensive treatment.

(d) *Summary*

We have developed a novel conceptual approach that can distinguish between density-dependent speciation and other processes that result in temporal declines in speciation rates. We do not claim that density dependence is the only possible explanation for our finding of temporally declining speciation in *Dendroica* wood warblers, but our results clearly eliminate two competing alternative scenarios. Patterns of lineage accumulation in

this group are inconsistent with a simple model in which speciation rates vary linearly as a function of time. Moreover, the explosive-early accumulation of lineages is more consistent with density-dependent diversification than an artefactual decline in rates attributable to incomplete taxon sampling.

Previous studies have found co-occurring wood warbler taxa to differ in both foraging niche and other ecological traits (e.g. MacArthur 1958; Morse 1989; Martin & Martin 2001), and we have previously shown that local warbler assemblages are phylogenetically overdispersed (Lovette & Hochachka 2006). These features suggest the possibility that wood warbler communities have been assembled through adaptive radiation (Gillespie 2004; but see Freckleton & Harvey 2006). Our finding that speciation rates in wood warblers show density dependence adds a novel dimension to our understanding of this continental radiation because it suggests the possibility of coupling between lineage and ecological diversification in this group.

REFERENCES

Baldwin, B. G. & Sanderson, M. J. 1998 Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proc. Natl Acad. Sci. USA* **95**, 9402–9406. (doi:10.1073/pnas.95.16.9402)

Barraclough, T. G. & Vogler, A. P. 2002 Recent diversification rates in North American tiger beetles estimated from a dated mtDNA phylogenetic tree. *Mol. Biol. Evol.* **19**, 1706–1716.

Barraclough, T. G., Hogan, J. E. & Vogler, A. P. 1999 Testing whether ecological factors promote cladogenesis in a group of tiger beetles (Coleoptera: Cicindelidae). *Proc. R. Soc. B* **266**, 1061–1067. (doi:10.1098/rspb.1999.0744)

Bernatchez, L., Chouinard, A. & Lu, G. 1999 Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. *Biol. J. Linn. Soc.* **68**, 173–194.

Cardillo, M., Huxtable, J. S. & Bromham, L. 2003 Geographic range size, life history and rates of diversification in Australian mammals. *J. Evol. Biol.* **16**, 282–288. (doi:10.1046/j.1420-9101.2003.00513.x)

Case, T. J., Holt, R. D., McPeek, M. A. & Keitt, T. H. 2005 The community context of species' borders: ecological and evolutionary perspectives. *Oikos* **108**, 28–46. (doi:10.1111/j.0030-1299.2005.13148.x)

Drummond, A. J. & Rambaut, A. 2007 BEAST: Bayesian evolutionary analysis sampling trees. *BMC Evol. Biol.* **7**, 214. (doi:10.1186/1471-2148-7-214)

Drummond, A. J., Ho, S. W. Y., Phillips, M. J. & Rambaut, A. 2006 Relaxed phylogenetics and dating with confidence. *PLoS Biol.* **4**, 699–710. (doi:10.1371/journal.pbio.0040088)

Erwin, D. H., Valentine, J. W. & Sepkoski, J. J. 1987 A comparative study of diversification events: the Early Paleozoic versus the Mesozoic. *Evolution* **41**, 1177–1186. (doi:10.2307/2409086)

Foote, M. 1996 Ecological controls on the evolutionary recovery of post-Paleozoic crinoids. *Science* **274**, 1492–1495. (doi:10.1126/science.274.5292.1492)

Freckleton, R. P. & Harvey, P. H. 2006 Detecting non-Brownian trait evolution in adaptive radiations. *PLoS Biol.* **4**, e373. (doi:10.1371/journal.pbio.0040373)

Gavrilets, S. & Vose, A. 2005 Dynamic patterns of adaptive radiation. *Proc. Natl Acad. Sci. USA* **102**, 18 040–18 045. (doi:10.1073/pnas.0506330102)

Gillespie, R. 2004 Community assembly through adaptive radiation in Hawaiian spiders. *Science* **303**, 356–359. (doi:10.1126/science.1091875)

Harmon, L. J., Schulte, J. A., Larson, A. & Losos, J. B. 2003 Tempo and mode of evolutionary radiation in iguanian lizards. *Science* **301**, 961–964. (doi:10.1126/science.1084786)

Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. 2008 GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131. (doi:10.1093/bioinformatics/btm538)

Hubbell, S. P. 2000 *The unified neutral theory of biodiversity and biogeography.* Princeton, NJ: Princeton University Press.

Irschick, D. J., Vitt, L. J., Zani, P. A. & Losos, J. B. 1997 A comparison of evolutionary radiations in mainland and Caribbean *Anolis* lizards. *Ecology* **78**, 2191–2203. (doi:10.1890/0012-9658(1997)078[2191:ACOERI]2.0.CO;2)

Jablonski, D. & Roy, K. 2003 Geographical range and speciation in fossil and living molluscs. *Proc. R. Soc. B* **270**, 401–406. (doi:10.1098/rspb.2002.2243)

Kozak, K. H., Weisrock, D. W. & Larson, A. 2006 Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders Plethodontidae: *Plethodon*. *Proc. R. Soc. B* **273**, 539–546. (doi:10.1098/rspb.2005.3326)

Levinton, J. S. 1979 A theory of diversity equilibrium and morphological evolution. *Science* **204**, 335–336. (doi:10.1126/science.204.4390.335)

Lovette, I. J. & Bermingham, E. 1999 Explosive speciation in the New World *Dendroica* warblers. *Proc. R. Soc. B* **266**, 1629–1636. (doi:10.1098/rspb.1999.0825)

Lovette, I. J. & Bermingham, E. 2002 What is a wood-warbler? Molecular characterization of a monophyletic parulidae. *Auk* **119**, 695–714. (doi:10.1642/0004-8038(2002)119[0695:WIAWWM]2.0.CO;2)

Lovette, I. J. & Hochachka, W. M. 2006 Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. *Ecology* **87**, S14–S28. (doi:10.1890/0012-9658(2006)87[14:SEOPNC]2.0.CO;2)

Lovette, I. J., Bermingham, E. & Ricklefs, R. E. 2002 Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. *Proc. R. Soc. B* **269**, 37–42. (doi:10.1098/rspb.2001.1789)

MacArthur, R. H. 1958 Population ecology of some warblers of northeastern coniferous forests. *Ecology* **39**, 599–619. (doi:10.2307/1931600)

Martin, P. R. & Martin, T. E. 2001 Ecological and fitness consequences of species coexistence: a removal experiment with wood warblers. *Ecology* **82**, 189–206. (doi:10.2307/2680096)

McPeek, M. A. & Brown, J. M. 2000 Building a regional species pool: diversification of the *Enallagma* damselflies in eastern North America. *Ecology* **81**, 904–920. (doi:10.2307/177166)

Morse, D. H. 1989 *American warblers: an ecological and behavioral perspective.* Cambridge, MA: Harvard University Press.

Nee, S. 2001 Inferring speciation rates from phylogenies. *Evolution* **55**, 661–668. (doi:10.1554/0014-3820(2001)055[0661:ISRFP]2.0.CO;2)

Nee, S. 2006 Birth–death models in macroevolution. *Annu. Rev. Ecol. Evol. Syst.* **37**, 1–17. (doi:10.1146/annurev.ecolsys.37.091305.110035)

Nee, S., Mooers, A. & Harvey, P. H. 1992 Tempo and mode of evolution revealed from molecular phylogenies. *Proc. Natl Acad. Sci. USA* **89**, 8322–8326. (doi:10.1073/pnas. 89.17.8322)

Nee, S., May, R. M. & Harvey, P. H. 1994 The reconstructed evolutionary process. *Phil. Trans. R. Soc. B* **344**, 305–311. (doi:10.1098/rstb.1994.0068)

Nylander, J. S. 2004 MRMODELTEST. See http://www.abc.se/wnylander/.

Phillimore, A. B. & Price, T. D. 2008 Density-dependent cladogenesis in birds. *PLoS Biol.* **6**, e71. (doi:10.1371/journal.pbio.0060071)

Price, T., Lovette, I. J., Bermingham, E., Gibbs, H. L. & Richman, A. D. 2000 The imprint of history on communities of North American and Asian warblers. *Am. Nat.* **156**, 354–367. (doi:10.1086/303397)

Pybus, O. G. & Harvey, P. H. 2000 Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc. R. Soc. B* **267**, 2267–2272. (doi:10.1098/rspb.2000.1278)

Rabosky, D. L. 2006a LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates. *Evol. Bioinform. Online* **2**, 257–260.

Rabosky, D. L. 2006b Likelihood methods for detecting temporal shifts in diversification rates. *Evolution* **60**, 1152–1164. (doi:10.1554/05-424.1)

Rabosky, D. L. & Lovette, I. J. In press. Explosive evolutionary radiations: increasing extinction or decreasing speciation through time? *Evolution*. (doi:10.1111/j.1558-5646.2008.00409.x)

Rabosky, D. L., Donnellan, S. C., Talaba, A. L. & Lovette, I. J. 2007a Exceptional among-lineage variation in diversification rates during the radiation of Australia's largest vertebrate clade. *Proc. R. Soc. B* **274**, 2915–2923. (doi:10.1098/rspb.2007.0924)

Rabosky, D. L., Reid, J., Cowan, M. A. & Foulkes, J. 2007b Community-wide overdispersion of body size in Australian desert lizard communities. *Oecologia* **154**, 561–570. (doi:10.1007/s00442-007-0849-1)

Rawlings, L. H., Rabosky, D. L., Donnellan, S. C. & Hutchinson, M. N. 2008 Python phylogenetics: inferences from morphology and mitochondrial DNA. *Biol. J. Linn. Soc.* **93**, 603–619. (doi:10.1111/j.1095-8312. 2007.00904.x)

Revell, L. J., Harmon, L. J. & Glor, R. E. 2005 Under-parameterized model of sequence evolution leads to bias in the estimation of diversification rates from molecular phylogenies. *Syst. Biol.* **54**, 973–983. (doi:10.1080/10635150500354647)

Ricklefs, R. E. & Cox, G. W. 1972 Taxon cycles and the West Indian avifauna. *Am. Nat.* **106**, 195–219. (doi:10.1086/282762)

Rosenzweig, M. L. 1995 *Species diversity in space and time*. Cambridge, UK: Cambridge University Press.

Rüber, L. & Zardoya, R. 2005 Rapid cladogenesis in marine fishes revisited. *Evolution* **59**, 1119–1127. (doi:10.1554/04-394)

Schluter, D. 2000 *The ecology of adaptive radiation*. New York, NY: Oxford University Press.

Seehausen, O. 2006 African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. B* **273**, 1987–1998. (doi:10.1098/rspb.2006.3539)

Sepkoski, J. J. 1998 Rates of speciation in the fossil record. *Phil. Trans. R. Soc. B* **353**, 315–326. (doi:10.1098/rstb.1998.0212)

Simpson, G. G. 1953 *The major features of evolution*. New York, NY: Columbia University Press.

Stanley, S. M. 1973 Effects of competition on rates of evolution, with special reference to bivalve molluscs and mammals. *Syst. Zool.* **22**, 486–506. (doi:10.2307/2412955)

Turgeon, J., Stoks, R., Thum, R. A., Brown, J. M. & McPeek, M. A. 2005 Simultaneous quaternary radiations of three damselfly clades across the Holarctic. *Am. Nat.* **165**, E78–E107. (doi:10.1086/428682)

Valentine, J. W. 1985 Biotic diversity and clade diversity. In *Phanerozoic diversity patterns* (ed. J. W. Valentine), pp. 419–424. Princeton, NJ: Princeton University Press.

Walker, T. D. & Valentine, J. W. 1984 Equilibrium models of evolutionary species diversity and the number of empty niches. *Am. Nat.* **124**, 887–899. (doi:10.1086/284322)

Weir, J. T. 2006 Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. *Evolution* **60**, 842–855. (doi:10.1554/05-272.1)