POSITIVE CORRELATION BETWEEN DIVERSIFICATION RATES AND PHENOTYPIC EVOLVABILITY CAN MIMIC PUNCTUATED EQUILIBRIUM ON MOLECULAR PHYLOGENIES

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The hypothesis of punctuated equilibrium proposes that most phenotypic evolution occurs in rapid bursts associated with speciation events. Several methods have been developed that can infer punctuated equilibrium from molecular phylogenies in the absence of paleontological data. These methods essentially test whether the variance in phenotypes among extant species is better explained by evolutionary time since common ancestry or by the number of estimated speciation events separating taxa. However, apparent "punctuational" trait change can be recovered on molecular phylogenies if the rate of phenotypic evolution is correlated with the rate of speciation. Strong support for punctuational models can arise even if the underlying mode of trait evolution is strictly gradual, so long as rates of speciation and trait evolution covary across the branches of phylogenetic trees, and provided that lineages vary in their rate of speciation. Species selection for accelerated rates of ecological or phenotypic divergence can potentially lead to the perception that most trait divergence occurs in association with speciation events.

In a seminal paper, Eldredge and Gould (1972) proposed that phenotypic evolution is characterized by long periods of stasis interspersed with bursts of trait change associated with speciation. This hypothesis of "punctuated equilibrium" catalyzed a significant body of research on the tempo and mode of phenotypic evolution using both paleontological and neontological data (Ricklefs 1980; Douglas and Avise 1982; Futuyma 1987). Punctuated equilibrium has had a significant and lasting influence on the study of phenomena as diverse as language (Atkinson et al. 2008), rates of molecular evolution (Pagel et al. 2006), and phenotypic trends in the fossil record (Hunt 2007).

Several methods have been developed that can detect punctuated equilibrium on molecular phylogenies (Pagel 1997; Mooers et al. 1999; Bokma 2002, 2008; Ingram 2011), where punctuated equilibrium is defined as a pattern of trait divergence associated with speciation events (e.g., "speciational" trait evolution). To a first approximation, these methods simply test whether the variance in phenotypes increases as function of time or as a function of the number of speciation events that have occurred.

Under a Brownian motion process, the distribution of phenotypes for a single character state across a phylogenetic tree will follow a multivariate normal distribution with mean equal to the trait value at the root node and variance–covariance matrix Σ. The phenotypic covariances between species are proportional to their shared evolutionary history, and the variances of species are proportional to the root-to-tip sums of branch lengths. However, if

punctuated trait evolution occurs during speciation, the expected variance should be proportional to the total number of speciation events that have occurred along each ancestor–descendant path from root to tips, and covariances between species should be proportional to the total number of "shared" speciation events that have occurred. As in the gradual case, distributions of phenotypes under speciational models are thus expected to be multivariate normal, but phenotypic variances and covariances should be a function of the number of speciation events that have occurred during the evolution of particular lineages. All things being equal, this leads to the expectation that—on average—proportionately greater phenotypic divergence should be observed among species in high diversity clades than in low diversity clades. We can use these expectations to develop a straightforward test for punctuated equilibrium on time-calibrated molecular phylogenies (Pagel 1997). In the simplest case, we simply maximize and compare the likelihoods of character state data under each of the multivariate normal distributions defined above, with two parameters estimated for each model: (1) the trait value at the root node in the phylogeny and (2) a scalar multiplier of the variance–covariance matrix.

Here, I demonstrate that positive correlations between the rate of trait evolution and the rate of speciation can lead to apparent speciational patterns of trait evolution on molecular phylogenies, even when there is no association between speciation events and trait change. When such correlations exist, proportionately greater amounts of phenotypic evolution can occur in regions of phylogenetic trees that have elevated rates of speciation. Species selection (Lloyd and Gould 1993; Jablonski 2008; Rabosky and McCune 2010) for lineages with the capacity for rapid phenotypic evolution may thus lead to phylogenetic distributions of character states that are difficult to distinguish from simple patterns of speciational trait evolution.

Materials and Methods

I tested whether a positive correlation between the rate of trait evolution and the rate of speciation could lead to the impression of speciational trait change on phylogenetic trees. I generated phylogenetic trees under a simulation algorithm that allowed rates of phenotypic evolution and speciation to "evolve" through time under a correlated random-walk process. Specifically, I assumed that the natural logarithm of the speciation rate λ and the phenotypic rate β evolved along the branches of a phylogenetic tree under a multivariate Brownian motion process with a variance–covariance matrix given by

$$\Phi = \begin{bmatrix} \sigma_\lambda^2 & \sigma_{\lambda\beta} \\ \sigma_{\lambda\beta} & \sigma_\beta^2 \end{bmatrix},$$

where σ^2 represents the variance of $\log(\lambda)$ or $\log(\beta)$ through time, and $\sigma_{\lambda\beta}$ represents their covariance.

Each simulation was initiated with a "root" speciation event, an evolutionary rate matrix Φ, and initial values for traits, speciation rates, and phenotypic evolutionary rates. The simulation algorithm repeated the following steps until the age of the process reached a predefined maximum time: (1) for each lineage with speciation rate λ_i and β_i, choose a waiting time T to the next speciation event from an exponential distribution with rate λ_i; (2) new values $\log(\lambda_k)$ and $\log(\beta_k)$ are sampled from a multivariate normal distribution with respective mean values of $\log(\lambda_i)$, $\log(\beta_i)$, and variance–covariance matrix $T\Phi$. Each phylogenetic tree thus contained a complete record of speciation and phenotypic rates at all internal and terminal nodes. Extinction rates were constrained to be equal to zero, such that reconstructed phylogenies contained a complete record of all speciation events that occurred during the simulation process. I ignored extinction to model phenotypes as a function of the true number of speciation events that have occurred, without introducing additional model parameters to estimate the number of "missing" (unobserved) events. Regardless, even under high extinction rates, the reconstructed nodal path lengths (root-to-tips) are expected to be highly correlated with true nodal path lengths that contain all unobserved speciation events (Rabosky and Lovette 2009).

To assess whether the simulation algorithm worked correctly, I tested whether the simulation model matrix Φ could be recovered from the variance–covariance structure of λ and β from simulated phylogenies. I estimated sample variance–covariance matrices for phylogenies simulated from 5000 random-valued Φ matrices. For each simulated phylogeny, I computed a series of contrasts in the logarithm of rates for the ith branch as

$$C_i = \frac{\log(R_{i,desc}) - \log(R_{i,parent})}{\sqrt{\Delta t_i}},$$

where $\boldsymbol{R_{i,desc}}$ and $\boldsymbol{R_{i,parent}}$ are vectors of rates $[\lambda, \beta]$ at descendant and parent nodes, respectively, and where Δt_i is the length of the ith branch. There are a total of $2N - 2$ such contrasts for a phylogenetic tree with N species. If the simulation algorithm is working correctly, these contrasts should be multivariate normally distributed with a mean of 0 and variance–covariance matrix Φ (Lartillot and Poujol 2011). Letting $\mathbf{C}$ denote an $n \times 2$ matrix of contrasts across all branches ($n = 2N - 2$), I estimated the sample variance–covariance matrix as $\mathbf{C^T C}/(n - 1)$. These analyses suggest that the variance and covariance components of evolutionary rates in simulated phylogenies reflect the underlying model matrix Φ (Fig. 1).

Given the vector of phenotypic rates at each internal and terminal node in the tree, I rescaled all branch lengths by the expected variance of the corresponding Brownian motion process. I assumed each branch was characterized by a phenotypic rate
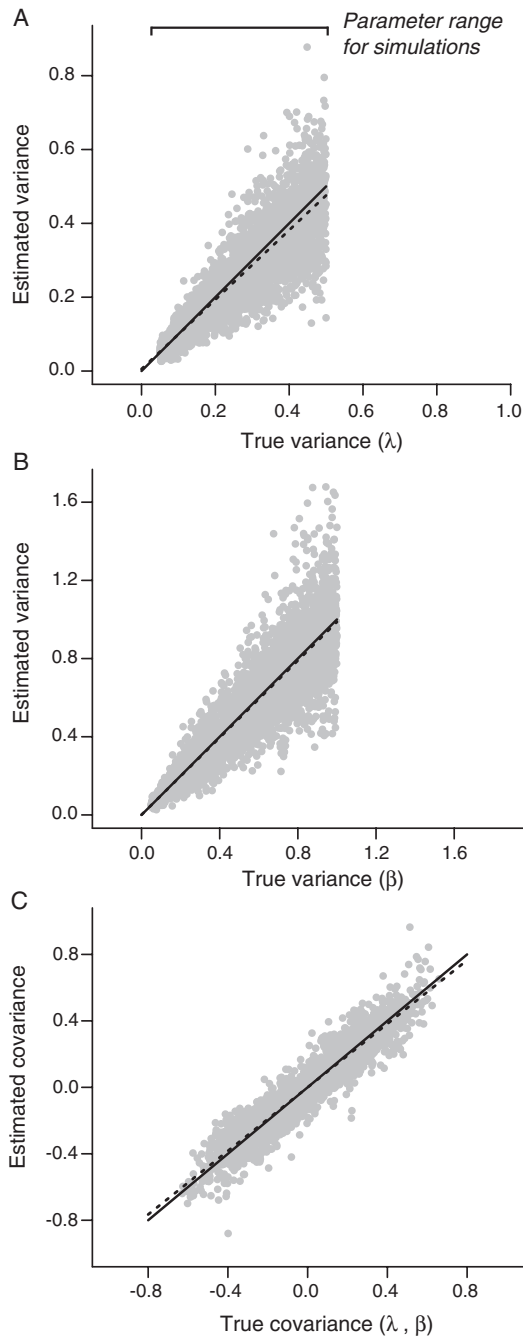
Figure 1. Variance components estimated from simulated datasets are highly correlated with true simulation parameters. **(A)** Rate of evolution of log-transformed speciation rate, σ_λ^2; **(B)** rate of evolution of log-transformed phenotypic evolutionary rate, σ_β^2; **(C)** covariance between $\log(\lambda)$ and $\log(\beta)$. Results are based on 5000 random Φ matrices. Simulation values of σ_λ^2 and σ_β^2 were sampled uniformly on (0.1, 0.5) and (0.1, 1.0), respectively, and covariances were computed from correlation coefficients drawn uniformly on (−1, 1). Dashed lines are observed ordinary least squares regression lines for the relationship between simulation values and estimates; solid lines denote expectation if estimates are exactly equal to simulation values.

that was equal to the mean of the rate at the parent and descendant nodes; the rescaled branch length was obtained by simply multiplying the observed branch length by this quantity. Phenotypic traits were simulated by sampling from a multivariate normal distribution with a zero-valued mean vector and a variance–covariance matrix computed from the rescaled phylogeny. All sets of traits simulated in this fashion conform to a strict gradual mode of trait evolution with branch-specific rates of trait evolution across the tree.

I used the method described by Ingram (2011) to test whether the observed phenotypic data could best be explained by simple models of gradual and speciational trait evolution. Although my analyses followed Ingram's (2011) formulation, this approach is closely related to Bokma's (2002) method for partitioning trait evolution into gradual and speciational components. The total variance of phenotypic change over a single branch of length t is

$$\sigma_{Total}^2 = \sigma_g^2 t + \sigma_p^2 s,$$

where σ_g^2 is the gradual variance component, σ_p^2 is the speciational component, and s is the number of speciation events occurring along the branch. Because I did not include extinction in the simulation model, I did not estimate the number of unobserved speciation events that might have contributed to the speciational variance; hence, the value of s was fixed at 1. For each simulated phylogeny, I used maximum likelihood to fit (1) a simple model of trait evolution by Brownian motion, and (2) the model described above with separate parameters for speciational and gradual components of the total phenotypic variance. Multiple optimizations were performed on each simulated phylogeny with random starting parameters.

I simulated phylogenies with trait data under 33 different Φ matrices. Rates of speciation and trait evolution at the root node were fixed at 0.2 and 0.5, respectively. The rate of evolution of the logarithm of the speciation rate, σ_λ^2, was held constant at 0.2 for all simulations. The rate of evolution of the (log) Brownian rate parameter (σ_β^2) was set to 0.25, 0.5, or 1.0. For each value of σ_β^2, the correlation between the (log) phenotypic evolutionary rates and the (log) rate of speciation was varied from −0.99 to 0.99 ($r = [−.99, −0.8, −0.6, −0.4, −0.2, 0, 0.2, 0.4, 0.6, 0.8, 0.99]$) and the covariance between rates ($\sigma_{\lambda,\beta}$) was computed as $r\sigma_\lambda\sigma_\beta$. Phylogenetic trees were simulated for 10.0 time units, and 1000 phylogenies were simulated for each Φ matrix.

I rejected all simulations that contained fewer than 50 species, and simulations were aborted if they generated more than 500 species at any point in the process. This latter step was taken to prevent memory overflow: if speciation rates became sufficiently high as to generate at least 500 species during a single simulation, the number of species would typically become unmanageably large ($N > 10^5$) toward the end of the simulation. All simulations
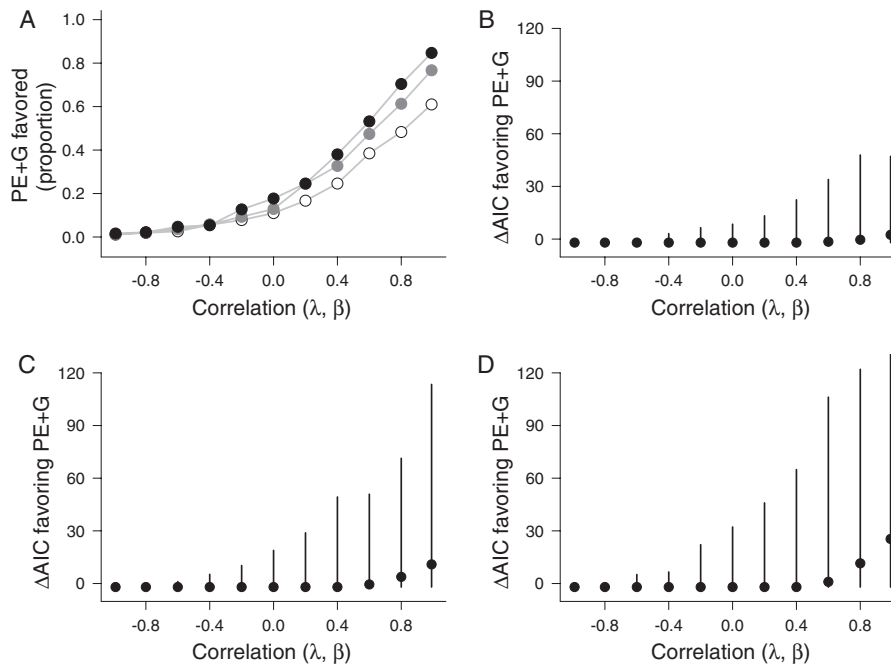
Figure 2. Combined speciational-gradual (PE+G) models fit simulated data better than strict gradual models when λ and β are positively correlated and support the punctuated equilibrium (PE) hypothesis, even when the true evolutionary model is gradual. (A) Proportion of simulations (*N* = 1000 per correlation), where AIC evidence favored a combined model over a gradual model (∆AIC > 0) as a function of the correlation between λ and β. White, gray, and black circles denote low (0.25), medium (0.5), and high (1.0) rates of evolution of the Brownian rate parameter β (σ_β^2). (B–D) Distribution of ∆AIC evidence favoring PE under $\sigma_\beta^2 = 0.25$ (B), $\sigma_\beta^2 = 0.50$ (C), and $\sigma_\beta^2 = 1.0$ (D). Shown are medians and 0.025 and 0.975 quantiles on the distribution of ∆AIC from simulations. Note that AIC evidence can lead to erroneous rejection of gradual models with a very high level of confidence under some simulation conditions. For example, the evidence ratio corresponding to ∆AIC = 5 in this case implies that a combined speciational-gradual model is approximately 12 times more likely than a pure gradual model, given the data; ∆AIC = 10 implies that a combined model is 150 times more likely than a gradual model.

and analyses were conducted in the R programming language (computer code is available as Supporting information).

Results and Discussion

When rates of trait evolution and speciation were uncorrelated or negatively correlated ($r \leq 0$), gradual models of trait evolution consistently outperformed a model with speciational and gradual components (Fig. 2A). Akaike information criterion (AIC) evidence overwhelmingly favored gradual models under all simulation scenarios considered with $r \leq 0$. However, a model that included speciational trait evolution was increasingly favored as the correlation between λ and β increased. When evolutionary rates were strongly and positively correlated, a majority of simulated datasets were best explained by a model that included both speciational and gradual components of trait evolution.

AIC evidence favoring a model with speciational trait evolution was extremely high under many simulation parameterizations (Fig. 2B–D; ∆AIC > 10), particularly under high rates of β evolution. For example, across all simulations with $r \geq 0.6$ and $\sigma_\beta^2 = 1$, more than 65% of all phylogenies were characterized

by ∆AIC > 5 in favor of the model with a speciational component (median: ∆AIC = 17.4), and the 0.95 quantile on the distribution of ∆AIC scores was 119.7. When a combined speciational-gradual model outperformed a strict gradual model, parameter estimates suggested that a substantial percentage of phenotypic variance resulted from speciational and not gradual processes of trait evolution (Table 1). Under strong correlations between λ and β, the proportion of phenotypic variance explained by speciational trait evolution approached 1 for many simulated datasets.

These results indicate that positive correlations between rates of trait evolution and rates of speciation can lead to the perception that trait change is associated with speciation events, as proposed under punctuated equilibrium hypothesis. This can occur even when the process of trait evolution is strictly gradual, provided that evolutionary rates of speciation and trait evolution are coupled across the branches of phylogenetic trees. The explanation for this phenomenon is simple. Methods for detecting punctuated equilibrium on molecular phylogenies rely on correlations between nodal path lengths (e.g., the number of nodes between the root and the tip of the tree) and total trait change. If this correlation is positive, it suggests that the number of intervening

Table 1. Proportion of total phenotypic variance explained by speciational component of trait evolution (Ingram's Ψ) for the subset of simulations where a combined speciational/gradual model explained the data better than a pure gradual model (see Fig. 2A). Shown are medians from the distribution of estimated proportions from each simulation scenario; numbers in parentheses denote 0.25 and 0.75 quantiles of the distribution of estimates).

Correlation (λ, β)	$\sigma_\beta^2=0.25$	$\sigma_\beta^2=0.5$	$\sigma_\beta^2=1.0$
0.2	0.07 (0.01, 0.22)	0.13 (0.04, 0.50)	0.23 (0.04, 0.83)
0.4	0.09 (0.03, 0.36)	0.22 (0.05, 0.74)	0.34 (0.06, 1.0)
0.6	0.14 (0.04, 0.41)	0.29 (0.06, 0.83)	0.46 (0.09, 1.0)
0.8	0.14 (0.04, 0.41)	0.33 (0.09, 0.89)	0.85 (0.28, 1.0)
1	0.26 (0.09, 0.57)	0.67 (0.24, 1.0)	1.0 (0.70, 1.0)

nodes between the root and the tip predicts phenotypic variation at the tip of the tree. If the rate of speciation is constant across the tree, such a correlation presumably reflects a speciational contribution to total trait variation. In the scenarios I have considered here, the rate of speciation is not constant across the phylogeny. If the rate of speciation covaries positively with the rate of trait evolution, phenotypic variances will also be correlated with nodal path lengths and speciational models of trait evolution can potentially fit the data better than gradual models, unless alternative models are considered that explicitly accommodate this variation in diversification rates.

These results do not imply that the inference of punctuated equilibrium from molecular phylogenies is generally invalid. Further work is needed to assess the biologically relevant range of parameter space to which the phenomenon documented here may be applicable. However, existing methods for modeling the relationship between trait change and speciation on molecular phylogenies assume that rates of species diversification and trait evolution are constant across the phylogeny. These assumptions may not be justified for some groups of organisms: rates of both species diversification and trait evolution vary substantially among lineages in many phylogenies (Rabosky and McCune 2010; Eastman et al. 2011; Revell et al. 2011). We do not yet have a methodological framework that can test whether coupling of these rates might explain patterns of speciational trait evolution observed on molecular phylogenies (Mattila and Bokma 2008; Ingram 2011). One possible way forward would be to use hierarchical phylogenetic models (Lartillot and Poujol 2011; Rabosky and Adams 2012) to explicitly estimate the covariance between rates of species diversification and rates of phenotypic evolution. It remains unclear whether such an approach would have sufficient power to distinguish between punctuated equilibrium and correlated rates of speciation and phenotypic evolution.

Some ecological models of adaptive radiation propose a coupling between rates of species diversification and rates of phenotypic evolution, with initially high evolutionary rates declining through time as ecological opportunity decreases (Schluter 2000;

Harmon et al. 2003; Mahler et al. 2010). This process will not necessarily generate speciational patterns of trait evolution on molecular phylogenies. Support for speciational models of trait evolution arises when total phenotypic change is correlated with root-to-tip nodal path lengths. If rates of species diversification and trait evolution have slowed through time (Mahler et al. 2010), there is no necessary reason why nodal path lengths should correlate with phenotypic evolutionary rates. Indeed, temporal coupling between rates of species diversification and phenotypic evolution can occur in the absence of any heterogeneity in the rate of species diversification among contemporaneous lineages. Such coupling would be unlikely to lead to patterns of speciational trait evolution on molecular phylogenies, at least via the mechanism discussed here. Nonetheless, it is possible that other evolutionary processes—in addition to evolvability–diversification correlations—can lead to similar patterns of speciational trait evolution on molecular phylogenies.

We are only beginning to understand the relationship between rates of species diversification and rates of phenotypic evolution (Adams et al. 2009; Mahler et al. 2010; Rabosky and Adams 2012), and it is possible that the phenomenon I report here may not be a problem for many or most species-level phylogenies. However, theoretical considerations suggest that evolutionary "versatility" or "evolvability" might be coupled to species diversification (Vermeij 1973; Kirschner and Gerhart 1998). For example, lineages with high phenotypic evolutionary rates might be able to diversify into an increased number of ecological niches (Adamowicz et al. 2008), thus diversifying at higher rates if there are strong ecological controls on species richness. This idea has a long history in the literature, and early discussions of "key innovations" proposed that extraordinary evolutionary radiations might be explicable in part by the evolution of traits promoting morphological and functional diversification (Liem and Osse 1975). Further research is needed to address the extent to which evolvability–diversification correlations have shaped large-scale evolutionary trends, but results presented here indicate that such relationships can potentially contribute to patterns of "punctuated equilibrium" on molecular phylogenies.

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