Phylogenetic Targeting of Research Effort in Evolutionary Biology

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Abstract: Many questions in comparative biology require that new data be collected, either to build a comparative database for the first time or to augment existing data. Given resource limitations in collecting data, the question arises as to which species should be studied to increase the size of comparative data sets. By taking hypotheses, existing data relevant to the hypotheses, and a phylogeny, we show that a method of “phylogenetic targeting” can systematically guide data collection while taking into account potentially confounding variables and competing hypotheses. Phylogenetic targeting selects potential candidates for future data collection, using a flexible scoring system based on differences in pairwise comparisons. We used simulations to assess the performance of phylogenetic targeting, as compared with the less systematic approach of randomly selecting species (as might occur when data have been collected without regard to phylogeny and variation in the traits of interest). The simulations revealed that phylogenetic targeting increased the statistical power to detect correlations and that power increased with the number of species in the tree, even when the number of species studied was held constant. We also developed a Web-based computer program called PhyloTargeting to implement the approach (http://phylotargeting.fas.harvard.edu).

Keywords: comparative method, phylogeny, correlated evolution, taxon sampling, pairwise comparison.

Introduction

The comparative method has played a major role in uncovering adaptive trait evolution in biological systems (Ridley 1983; Harvey and Pagel 1991; Pagel 1999; Martins 2000). The comparative method has, for example, revealed links between mating systems and sperm competition in primates (Harcourt et al. 1981) and other animals (Møller 1991; Hosken 1997). The comparative method also supports a model of sexual selection in which females choose males on the basis of their ability to resist parasites (Hamilton and Zuk 1982), and it has been used to probe the origins of both parasitic and symbiotic associations (e.g., Hugot 1999; Lutzoni et al. 2001). More recently, comparative methods have been applied to study phylogenetic community ecology (Webb et al. 2002), for example, in the context of the phylogenetic overdispersion of mammalian communities (Cooper et al. 2008). The comparative method can also be used to address conservation issues (Fisher and Owens 2004), such as questions involving the factors that influence rates of extinction (Purvis et al. 2000b) and how the phylogenetic clumping of conservation threat status can lead to greater loss of phylogenetic diversity when species become extinct (Purvis et al. 2000a).

A comparative analysis requires data on a set of species relevant to a hypothesis of interest. Usually, however, data are available for only a fraction of the species in a clade, and data collection in both the field and the laboratory is expensive and time consuming. A proper selection of species to study is a nontrivial and multifaceted problem (Garland 2001; Westoby 2002) that has rarely been addressed in a systematic way. Instead, species are often chosen either randomly or subjectively (Westoby 1999; Faustino et al. 2010) because they are of “particular (and perhaps irrational) interest” (Garland 2001, p. 119). Two problems are introduced when species are chosen in an unsystematic way. First, the full range of variation is not used to test the hypotheses. Second, taxonomic gap bias may occur, meaning that data collection has been focused on a few “popular” lineages. These different kinds of biases—incomplete variation and gap biases—can make a momentous difference to the conclusions one draws. In studies of primates, for example, results of comparative research are likely to change when the sample is tilted toward terrestrial species rather than those that live in trees, because terrestrial species possess larger body masses, exhibit different locomotor patterns, and live in larger social groups.
To address these issues, methods are required that quantify potential biases in comparative data sets and identify the species that should be studied in the future. Indeed, it is common to read in articles of comparative research that further sampling is required to validate the findings, because either the sample sizes were small or the sample was biased toward particular species within a clade (e.g., in the study of sleep patterns; Roth et al. 2006; Capellini et al. 2009; Nunn et al. 2009). Unfortunately often, however, only general guidelines for this selection process have been given, and these guidelines are often specific to the question of interest (Westoby 2002). To our knowledge, no method yet exists that is flexible and specific enough to address the crucial task of prioritizing future research in light of specific hypotheses about the apportionment of variation in relation to one or more ecological factors.

Only a handful of studies have investigated ways of systematically identifying species to study. For example, Ackerly (2000) compared the performance of different taxon sampling strategies and found their statistical performances to differ substantially. One of the algorithms he examined is based on the pairwise comparison approach (Felsenstein 1985, p. 13; Møller and Birkhead 1992; Oakes 1992; Read and Nee 1995; Purvis and Bronham 1997; Maddison 2000) and identifies meaningful comparisons by selecting species pairs that differ by a certain amount in the independent variable, following the suggestion of Westoby (1999). Although it overestimates the magnitude of the correlation, Ackerly (2000) showed that this design increases the statistical power to detect correlated evolution (see also Garland 2001). Major weaknesses of the method are that the threshold for when differences are large is arbitrary, that it is dependent on the data set, and that it must be set manually, which limits its applicability considerably. Mitani et al. (1996) considered sampling strategies in relation to testing competing hypotheses, while Read and Nee (1995) discussed the need to identify pairs that contribute for or against hypotheses. Similarly, Maddison (2000) presented a methodology for choosing species pairs in which each pair is “a comparison relevant for the question of interest” (p. 198). However, his method is designed for binary rather than continuously varying data and can handle only fully bifurcating trees, and thus it does not provide enough flexibility for identifying meaningful comparisons with real data.

The method of pairwise comparisons has been used frequently to identify meaningful comparisons. Several reasons exist for using pairwise comparisons. For example, the method of pairwise comparison relies on fewer assumptions (Ackerly 2000; Maddison 2000; Hearn and Huber 2006) than other methods. Thus, unlike phylogenetically independent contrasts (PIC; Felsenstein 1985; Harvey and Pagel 1991; Garland et al. 1992), pairwise comparison does not require a specific model of evolution or the estimation of states at interior nodes. In addition, some sets of species within a larger clade might not be directly comparable in standard implementations of comparative methods, such as PIC. In regard to mammalian sleep, for example, some cetaceans sleep with only one-half of their brains (Lyamin et al. 2008), making it difficult to compare the measurements of sleep in cetaceans with those in other mammals. The method of selecting specific pairwise comparisons provides a way to limit comparisons so that cetaceans are compared only with other cetaceans and noncetaceans are compared only with noncetaceans. Similarly, some behavioral experiments might require similar sensory modalities or cognitive ability among species in the data set. Pairwise comparisons of some close relatives may be more appropriate for selecting species for focused comparative experiments that take these factors into account.

When using the method of pairwise comparisons, it is important that all pairs are phylogenetically independent, that is, that no branches are shared among the comparisons (Felsenstein 1985; Maddison 2000). In figure 2, for example, different sets of phylogenetically independent pairs (which we call a “pairing”; see Maddison 2000) are shown for each tree. Thus, when selecting phylogenetically independent pairs, the selection of a particular pair constrains which other pairs can be selected.

Here we present a new approach, which we call “phylogenetic targeting,” to systematically identify the species to be studied. Phylogenetic targeting is a taxon sampling approach that aims to prioritize future research by identifying species that should be studied in a target-oriented way under consideration of the specific hypotheses and data. It is not a new way to analyze comparative data or a substitute for existing analysis methods, but rather it draws on existing methods in comparative biology. This method uses the pairwise comparison approach and is based on a scoring system that incorporates phylogeny and data on variables relevant to testing hypotheses, specifically involving the predictor and response variables in a comparative test. The predictor variables can include potentially confounding variables or variables relevant to testing alternative hypotheses for an association. If external information suggests that comparisons should be restricted taxonomically or in relation to existing data, one can use the method to limit the species to be compared.

After assigning a score for each pair of species, phylogenetic targeting uses a newly developed algorithm to select the set of phylogenetically independent pairs of species that offer greater statistical power to test the hypothesis when data have been collected on the dependent variable.
After data collection, pairwise contrasts for the targeted species pairs can be used to test hypotheses, or one can use standard comparative techniques for testing correlated character evolution (fig. 1). This decision is up to the investigator and depends on the actual hypotheses, data, and analysis preferences (see “Discussion”). We use computer simulations to assess the degree to which phylogenetic targeting, compared with random sampling of species, increases statistical power for detecting correlated trait evolution. We have also implemented the method online (http://phylotargeting.fas.harvard.edu). We anticipate that the general approach developed here for pairwise comparisons can be adjusted for use with additional comparative methods, such as PIC or generalized least squares approaches, and we discuss some of these potential extensions.

Methods

The method requires a phylogeny and one or more explicit hypotheses that offer predictions for how variation in one trait ($X_1$) correlates with variation in another trait that is common to all the species, is the “target” of the analysis ($Y_t$; fig. 1). We call this association between $Y_t$ and $X_1$ the primary hypothesis. Additional hypotheses, if desired, are implemented through traits $X_2 … X_n$, which relate to competing hypotheses or potentially confounding variables. The goal of the method is to identify species that should be studied, with regard to $Y_t$, through the use of phylogenetic relationships and data already collected for the $X$ traits. Thus, a species cannot be included in a phylogenetic targeting analysis if data on the $X$ trait are lacking for that species.

We assume that larger evolutionary changes in $X_1$ provide higher statistical power for comparative tests to test the hypotheses, because they increase the available range of variation (Westoby et al. 1998; Westoby 1999; Garland 2001; Garland et al. 2005). We also assume that the characters show a linear relationship. Different targeting analyses are likely to focus on a primary hypothesis and various combinations of alternative hypotheses, and both discrete and continuous traits can be used. Scores, such that higher values indicate more preferred species to study, are calculated on the basis of user-defined criteria involving control of confounding variables, testing of alternative hypotheses, and availability of data on $Y_t$ for one or more species in a clade.

Calculating Pairwise Comparisons

The analysis starts by calculating all possible $n \times (n - 1)/2$ pairwise comparisons. In the tree shown in figure 2, for example, 15 comparisons can be constructed. The method as such does not rely on using only pairs of sister species, as pairs of more distantly related species could also offer compelling tests of the hypotheses (Read and Nee 1995; Westoby 1999; Maddison 2000). Pairwise comparisons with missing data in any of the traits except $Y_t$ are excluded. In addition, certain species can be excluded manually from the analysis, for example, in cases where an experiment can be applied to only certain species on the tree.

![Flow chart for applying phylogenetic targeting. Phylogenetic targeting is essentially a taxon sampling technique to systematically guide future data collection.](http://phylotargeting.fas.harvard.edu)
Calculating Scores for Models with a Single Predictor ($Y_t$ and $X_1$)

For predictions that involve only a primary hypothesis (i.e., only one independent variable), phylogenetic targeting uses a scoring system that maximizes the variability in $X_1$. In other words, species pairs that differ the most in $X_1$ are targeted. If we were interested in hypotheses that involve body mass as an independent variable, for example, phylogenetic targeting gives pairs with the largest differences in body mass higher scores. Thus, pairwise comparisons with large differences in $X_1$ are scored higher, whereas smaller differences are scored less positively. These contrasts are then standardized to a scale of 0 to 1, with a difference of 0 assigned a score of 0 and the largest difference in all considered pairs assigned a score of 1. Note that even if no zero contrasts are found in the data, the method fixes the lowest contrast as 0. All other differences are assigned a score between 0 and 1 by applying a linear scaling transformation. We call this the score of $X_1$. If $X_1$ is an unordered discrete character, then the score will be either 0 or 1 regardless of the actual difference in character state assignments, whereas the difference is scored on an interval between 0 and 1 in the case of an ordered character, with the maximum number of character steps scored as 1.

Calculating Scores for Models with Covariates ($Y_t$, $X_1$, $X_2$, ..., $X_n$)

Models that incorporate additional traits enable the testing of different kinds of hypotheses (e.g., mutually exclusive and non-mutually exclusive), and they can be used to control for confounding variables. For each $X_2$ ... $X_n$, a separate scoring mechanism is defined in which larger contrasts have either a negative or a positive influence on the overall score. The decision as to whether larger differences in each of the variables $X_2$--$X_n$ are scored higher or lower depends on whether the variables reflect confounding variables or a desire to distinguish among competing hypotheses. To simplify discussion, we consider a case in which only one additional variable is included; that is, $Y_t = f(X_0, X_1)$. Further details on the specifics of scoring are given below.

To control for confounding variables, the goal is to minimize variation in the predictor variable that corresponds to the confounding variable of interest, that is, $X_2$. Thus, pairwise comparisons in $X_2$ that make the absolute value of change in a particular confounding variable as small as possible are scored higher, whereas pairwise comparisons with larger differences are scored lower (score$_{NC}$, i.e., the score from standardizing the covariate for "no change"). The smallest pairwise contrast is assigned a score of 1, whereas the maximum pairwise contrast is assigned a score of 0. All other differences are assigned a score between 0 and 1.

To address mutually exclusive hypotheses, the goal is to maximize scores for $X_2$ that differ maximally from contrasts in $X_1$. Two different scoring options can be applied that both target large differences but differ in how they score these differences. The first option positively scores the differences in $X_2$ that are in the opposite direction from the difference in $X_1$, and it negatively scores differ-
ences in the same direction as \( X_i \) (score\(_{OD} \), i.e., the score from standardizing the covariate in the opposite direction). The largest difference in the opposite direction is assigned a score of 1, whereas the largest difference in the same direction is assigned a score of \(-1\). A difference of 0 is assigned a score of 0. In analogy to models with a single predictor, the method fixes the lowest contrast as 0, even if no zero contrasts are found in the data. This ensures that all nonzero differences are assigned a score that is different from 0. All other differences are assigned a score between \(-1\) and 1 by applying a linear scaling transformation, which is calculated separately for positive and negative contrasts. The second option is the opposite of the first option; that is, differences in the opposite direction from the difference in \( X_i \) are scored negatively and differences in the same direction are scored positively (score\(_{SD} \), i.e., the score from standardizing the covariate in the same direction). For example, this option might be useful if an increase in \( X_i \) is predicted to reduce \( Y \) and an increase in \( X_i \) is predicted to increase \( Y \). Thus, it is necessary to give higher scores to contrasts in the same direction for \( X_i \) and \( X_j \) in order to distinguish among the hypotheses.

For models with covariates, the direction of change for \( X_i \ldots X_j \) always refers to the direction of change in \( X_i \); for example, a positive value means that the direction of change is the same as in \( X_i \). By doing so, we force the difference in \( X_i \) (\( \Delta_{raw} \); see table 1) to be positive and achieve consistency with other widely used programs, such as CAIC (Purvis and Rambaut 1995) and PDAP-Mesquite (Midford et al. 2005). This “positivization assumption” also helps to make sense of the other trait differences and their directions when using the computer program, as it becomes possible to determine whether other pairwise comparisons are consistently positively or negatively associated with \( X_i \) (e.g., if \( X_i \) is positive, then it must be in the same direction as \( X_j \)). Although this is not strictly necessary for the algorithms implemented here, it helps to guide manual selection of contrasts in the Web-based implementation of phylogenetic targeting.

**Table 1: Illustration of the scoring system and the maximal pairing**

<table>
<thead>
<tr>
<th>Pairwise comparison</th>
<th>( X_i )</th>
<th>( X_j )</th>
<th>Summed score</th>
<th>Sum of branch lengths</th>
<th>Standardized summed score</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1-s2(^*)</td>
<td>.5</td>
<td>.385</td>
<td>-3</td>
<td>.831</td>
<td>-1.71</td>
</tr>
<tr>
<td>s1-s3</td>
<td>.8</td>
<td>.615</td>
<td>-1.5</td>
<td>.916</td>
<td>-0.86</td>
</tr>
<tr>
<td>s1-s4</td>
<td>1.3</td>
<td>1</td>
<td>-2.7</td>
<td>.848</td>
<td>-1.54</td>
</tr>
<tr>
<td>s1-s5</td>
<td>1</td>
<td>.769</td>
<td>14.8</td>
<td>.169</td>
<td>.831</td>
</tr>
<tr>
<td>s2-s3</td>
<td>.3</td>
<td>.231</td>
<td>1.5</td>
<td>.916</td>
<td>.084</td>
</tr>
<tr>
<td>s2-s4</td>
<td>.8</td>
<td>.615</td>
<td>1</td>
<td>.983</td>
<td>.017</td>
</tr>
<tr>
<td>s2-s5</td>
<td>.5</td>
<td>.385</td>
<td>17.8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>s2-s6</td>
<td>.1</td>
<td>.077</td>
<td>12.6</td>
<td>.292</td>
<td>.708</td>
</tr>
<tr>
<td>s3-s4(^*)</td>
<td>.5</td>
<td>.385</td>
<td>-1.2</td>
<td>.933</td>
<td>-0.69</td>
</tr>
<tr>
<td>s3-s5</td>
<td>.2</td>
<td>1.54</td>
<td>16.3</td>
<td>.084</td>
<td>.916</td>
</tr>
<tr>
<td>s3-s6</td>
<td>.2</td>
<td>1.54</td>
<td>-11.1</td>
<td>.376</td>
<td>-.634</td>
</tr>
<tr>
<td>s4-s5</td>
<td>.3</td>
<td>.231</td>
<td>-17.5</td>
<td>.017</td>
<td>-1</td>
</tr>
<tr>
<td>s4-s6</td>
<td>.7</td>
<td>.538</td>
<td>-12.3</td>
<td>.309</td>
<td>-.703</td>
</tr>
<tr>
<td>s5-s6(^*)</td>
<td>.4</td>
<td>.308</td>
<td>5.2</td>
<td>.708</td>
<td>-.292</td>
</tr>
</tbody>
</table>

Note: As applied to figure 3. \( \Delta_{raw} \) = raw difference of trait values (see fig. 3). See scoring section for details on score\(_{OD} \), score\(_{SD} \), and score\(_{NC} \). Calculation of the summed score was based on the score of \( X_i \) and the score\(_{NC} \) scoring option for \( X_j \); sum of branch lengths was calculated according to the tree in figure 2.

\(^*\) Pairs that are selected in the maximal pairing.
land et al. 1992). We call this the standardized summed score. In this score, all pairwise comparisons have a common variance, which is required by most statistical tests (see also “Discussion”).

Table 1 summarizes and applies the scoring system to the data set in figure 3, on the basis of controlling for $X_2$ as a confounding variable (score$_{NC}$). Different standardized summed scores would be obtained if we treated $X_2$ as representing a competing hypothesis and depending on the expected direction of $X_2$ in the context of competing hypotheses (see data for score$_{SD}$ and score$_{OD}$ in table 1).

**Availability Variable**

In addition to manually excluding species from an analysis, it is possible to define an “availability variable” to automatically exclude species or pairs in relation to the availability of data for $Y_t$. One can thus use the availability variable to identify other species that should be studied in the context of existing data on $Y_t$. An availability variable also provides a way to quickly pinpoint where the missing data points are in a phylogenetic context, which can help to identify biases in the distribution of the studied species.

The availability variable must be a discrete binary variable that identifies whether data are available for $Y_t$ for a particular species. For example, consider the scenario in figure 3, in which $B_t$ is the availability variable. Possible options would be to consider only pairs where data are available for both species in the pair (exclusion of all pairs except s1-s5), for one species in the pair (exclusion of s1-s5 and all combinations of s2, s3, s4, and s6), for at least one species in the pair (as when data are available for one species, but also including s1-s5), and for neither of the species in the pair (exclusion of the nine pairs with s1 and s5). Thus, this scoring procedure can be used in a variety of ways. For example, if the availability variable indicates that data are available for only a fraction of the species, then the majority of the pairs will be excluded if the option is chosen to consider only pairs where one species has already been studied and data are needed for the other species. In such a case, the pairs remaining are those containing one studied species and one that has yet to be studied. It can thus be seen as an additional selection factor that effectively constrains the species to be targeted.

**Maximal Pairing Algorithm**

The actual selection of species is performed by a dynamic programming algorithm that we call maximal pairing. The maximal pairing algorithm is a general optimization algorithm that selects pairs of species that are phylogenetically independent (see also Arnold and Stadler 2010). In contrast to PIC, where pairs can also involve internal nodes on the tree, the maximal pairing algorithm selects only

<table>
<thead>
<tr>
<th>s1</th>
<th>s2</th>
<th>s3</th>
<th>s4</th>
<th>s5</th>
<th>s6</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>-0.4</td>
<td>0.1</td>
<td>0.4</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>X2</td>
<td>10.5</td>
<td>7.5</td>
<td>9.0</td>
<td>7.8</td>
<td>25.3</td>
</tr>
<tr>
<td>Yt</td>
<td>5.5</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>6.9</td>
</tr>
<tr>
<td>Bt</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 3:** Example data set and phylogeny for applying phylogenetic targeting. The tree shows continuously varying traits $X_1$, $X_2$, and $Y_t$ and a binary trait $B_t$ indicating whether the species has already been studied in relation to $Y_t$. Two species have already been studied regarding $Y_t$, and data on $Y_t$ are missing for four species. The goal is to identify which of the four unstudied species should be targeted for studying $Y_t$. 

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pairs between the tips of the tree. The selection of pairs is based on the summed score for each pair, and the algorithm determines the set of phylogenetically independent pairs that maximizes the sum of the individual summed scores (table 1). This criterion is therefore assumed to maximize the power to test the hypotheses, given constraints on maintaining phylogenetic independence. With large data sets, it is difficult to find the maximal pairing manually, due to the large number of possible pairings and the complex phylogenetic dependence of pairs that must not share a branch (fig. 2). Despite some differences that involve execution time and representation of polytomies, the maximal pairing algorithm also works for polytomous trees (see app. A in the online edition of the American Naturalist for more details).

For models that involve only \( X \), for example, maximal pairing generally selects pairs of closely related species that maximize differences in \( X \); these pairs are often distantly related to the other pairs that are selected. In a comparative test, such a design is considered to be especially powerful (Garland et al. 2005). If, however, an additional trait \( X \) is used to control for confounding variables (thereby scoring small differences in \( X \) that are opposite in sign to corresponding differences in \( Y \)), then the algorithm both maximizes differences in \( X \) and minimizes differences in \( Y \). Conversely, if one aims to maximize differences in \( X \) (thereby scoring larger differences in \( X \) that are opposite in sign to \( X \) higher with score \( \Delta x_{nc} \)), then the algorithm maximizes differences in \( X \) and minimizes differences in \( X \). For models that involve only \( X \), the second variable was assumed to be \( Y \). We also standardized the scores. The maximal pairing was then calculated, and we selected the six highest-scoring pairs. We also randomly selected six phylogenetically independent pairs. To investigate whether the number of selected pairs impacts statistical performance, all analyses were repeated with 9 and 12 pairs.

To evaluate the statistical properties of both sampling approaches, we performed standard statistical tests on the basis of the selected pairwise comparisons. For this we used the character differences for \( X \) and \( Y \) for the selected pairs and then standardized them by their expected variance (square root of the sum of the branch lengths that connect the two species). We tested for an association between the two characters based on correlation through the origin (Garland et al. 1992), using a \( t \)-test with \( N - 2 \) degrees of freedom and \( \alpha = 0.05 \). We determined Type I error rates (incorrectly rejecting a true null hypothesis of no association between traits) and statistical power (probability of rejecting a false null hypothesis) for both sampling approaches. Type I error rates were calculated as the proportion of significant results based on \( P = .05 \) for data sets in which \( r = 0 \), while statistical power was based on the proportion of significant results for data sets in which \( r = 0.5 \).

In addition to tests based on pairwise comparisons, we performed tests based on the full set of independent contrasts. We did this because many users may be interested in using a full set of contrasts, yet the method operates by examining pairwise comparisons. Thus, understanding the statistical performance of phylogenetic targeting when it is used with PIC is an important step and expands its application spectrum. After pruning the tree to the subset of the selected pairs, we calculated PIC (Felsenstein 1985), using the APE package (Paradis et al. 2004). We tested for
a significant correlation between the two characters, using the methods described above.

We also tested how the inclusion of randomly selected, nontargeted species affects the results. This simulates a common situation because data are often already available for some species but missing for others. Specifically, we examined how including \( k \) random species affects the results for tests based on pairwise comparisons and PIC (with values of \( k \) ranging from 2 to 10 in steps of 2). We included these additional species from the set of species that were not selected by phylogenetic targeting (and thus without using the availability variable).

Finally, we analyzed how much of the original range of variation in the simulated data was available after the data were pruned to the selected species. This gives insights to the range of variation that is available for hypothesis testing under the two sampling techniques.

Results

PhyloTargeting Program

We created a freely available computer program, PhyloTargeting, that implements the phylogenetic targeting approach. It is Web based, taking the data as a Nexus file (Maddison et al. 1997) and providing a user-friendly, interactive, step-by-step interface, a variety of analysis options, and graphical visualizations of the results. The program is publicly available at http://phylotargeting.fas.harvard.edu.

Simulations

The simulations revealed that phylogenetic targeting substantially increases the range of biological variation that is sampled relative to random sampling (fig. 4). Phylogenetic targeting also provided substantially higher statistical power for detecting a true relationship (fig. 5). This held for both the pairwise tests and tests based on PIC. For the pairwise tests, Type I error rates for \( \alpha = 0.05 \) were elevated if the number of selected pairs was small, but they decreased to the expected level when more pairs were selected. For the tests based on PIC, Type I error rates were close to the expected level in all scenarios. Importantly, Type I error rates under random sampling and phylogenetic targeting were generally indistinguishable. All simulation results (including the results not highlighted in the article) are provided in an Excel file titled “Simulation Results,” available in the online edition of the American Naturalist.

Increasing the number of pairs that are selected by the sampling algorithms increased statistical power, as was expected (fig. 5). For the pairwise tests, it also decreased Type I error rates. The number of taxa per tree, however, revealed a more surprising effect. Even when the number of pairs was held constant, the statistical power increased with the number of taxa in the clade under phylogenetic targeting, and Type I error rates did not increase (fig. 5). When species were selected randomly, however, power did not increase with increasing clade size.

When the true correlation was 0.5, mean values of \( r \) were elevated; moreover, they increased with the number of species per tree (see “Simulation Results” Excel file). Thus, a sampling regime based on phylogenetic targeting resulted in biased estimates of evolutionary trait correlations when \( r \neq 0 \), whereas a random selection of species resulted in no bias. Importantly, however, no bias was found when the true correlation was 0, as is shown in the results for Type I error rates. Furthermore, the bias decreased when additional, randomly selected species were included (see also “Discussion”).

The results highlighted above are for a Brownian-motion process of character evolution. For the alternative models that we tested (see app. B), many of the results were comparable. However, for most of these analyses, Type I error rates were highly elevated and statistical power was reduced under the two sampling approaches and for PIC on the full tree (which we used as a control). Not surprisingly, the pairwise tests showed substantially less elevated Type I error rates if model assumptions were vi-
Figure 5: Selected results from the simulations under Brownian motion. Type I errors and statistical power for correlation tests based on pairwise comparisons (PC) and phylogenetically independent contrasts (PIC) are shown for phylogenetically targeted (PT) and random (R) taxon sampling. The first three bars in each category represent Type I error rates (based on 50-, 70-, and 90-species trees), and the last three bars represent statistical power (also based on 50-, 70-, and 90-species trees). Contrasts were standardized, and six pairs were selected.

Discussion

Comparative studies generally make use of available data. Here we show that the comparative approach can also be used to target species for future data collection. By applying the phylogenetic targeting concept, we can identify species that offer higher power to test predictions of a comparative hypothesis. Moreover, phylogenetic targeting provides a way to control for confounding variables when selecting species for further study or when testing competing hypotheses. The method will most likely be used to augment existing data, but it can also be applied to generate new data sets in the context of finite resources for data collection.

A major strength of the approach is that phylogenetic information is incorporated when selecting species to study (Garland 2001; Garland et al. 2005), thereby ensuring that the selected pairs are phylogenetically independent of one another. This makes it possible to analyze the data with standard statistical methods (i.e., pairwise tests). However, the simulations revealed that, when compared with PIC, statistical power here is reduced (see also Ackerly 2000). This may be due to the fact that for pairwise differences, the number of data points is reduced by a factor of approximately 2 because only the tips of the tree and not the interior nodes are contrasted. Furthermore, the bias in estimating the correlation coefficient is increased with pairwise comparisons. We therefore advise users to analyze the selected species with standard comparative methods on the basis of the full set of contrasts whenever possible, instead of using the differences for the selected pairs directly.

The simulation results revealed that phylogenetic targeting, compared with a random selection of species, provides many advantages for detecting correlated trait evolution. Statistical power was strongly increased in all of the cases we examined. Phylogenetic targeting used a higher percentage of the available range of variation for a character than did random sampling of species. We can therefore be more certain that the pattern holds generally across the clade of organisms rather than, for example, only among the species that are larger in body size or more amenable to study. Surprisingly, the simulations also revealed that statistical power increased with the number of species per tree, even when the number of taxa selected for study remained constant. Type I errors, however, were always close to the nominal level and were indistinguishable between phylogenetic targeting and random species sampling. Thus, applying the method to larger clades resulted in increased power without increasing the number of pairs examined, probably because having more taxa increased the magnitude of the differences that could be
selected overall (which in turn increased the ability to detect a correlation).

Phylogenetic targeting should be used with caution when one wants to determine the magnitude of a correlation. Like the pairwise approach of Westoby (1999), it overestimates the correlation coefficient (Ackerly 2000). This was true for both the pairwise tests and PIC, and the bias was stronger with the pairwise tests. The simulations also revealed that this overestimation increases with the number of species per tree, thus mirroring the increase in power. In the context of applying the method to real-world data in which data for $Y_i$ are already available for some of the species, however, simulations confirmed that this bias decreases substantially with the number of randomly selected species for which data are already available. For most questions of interest that we envision, data are often available on $Y_i$ for a number of species that often comprised a majority of the species in the data set. When such data are available, inclusion of already available data in subsequent analysis after applying phylogenetic targeting is highly recommended. Alternatively, users can implement the availability variable option described above to more fully integrate decisions about future data collection with already-studied species. Furthermore, as noted above, the bias is likely to decrease if additional traits representing confounding variables or alternative hypotheses are included in the analysis.

A few limitations and assumptions of phylogenetic targeting should be noted. Although the maximal pairing selects the set of species pairs that have the highest overall scores according to a user-defined scoring model, it may select species that are not directly comparable in relation to a particular test, such as an experiment that involves testing cognitive abilities. To overcome this possible weakness, our PhyloTargeting program provides a way for the user to manually select pairs in which particular comparisons are possible and to exclude other comparisons. Phylogenetic targeting must be used with caution if nonlinear relationships exist between the variables, and we advise users to critically examine the variables beforehand. Other critical issues are the phylogenetic tree, the representation of polytomies (see app. B), and the branch lengths on which the species selection is based. The selection of species can vary substantially between similar tree topologies due to the fact that the maximal pairing algorithm strictly maximizes the overall score, which can sometimes be heavily influenced by the topology. Branch lengths are assumed to be proportional to the expected variance in the amount of evolutionary changes along each branch (Brownian motion); this assumption becomes important in both phylogenetic targeting and subsequent analyses, particularly for PIC. If these assumptions are violated, Type I error rates are inflated and statistical power is reduced (Díaz-Uriarte and Garland 1996; Quader et al. 2004). Indeed, the simulations confirmed this effect; for almost all of the alternative models, Type I error rates were highly elevated. The only exception is the early-burst model, which yielded results that were very similar to those for Brownian motion (“Simulation Results” Excel file).

Because sister taxa will tend to be similar in many ways, confounding variables are expected to be less of a problem in comparisons of sister species (Harvey and Pagel 1991; Möller and Birkhead 1992). In our approach, however, more distantly related species pairs can also be selected. This can be critical, because other unmeasured, confounding variables may be introduced to the analysis. The comparison of distantly related species is comparable to an experiment with multiple uncontrolled variables (Garland and Adolph 1994; Garland 2001). The more distantly related two species are, the more likely it is that such an effect could bias the results. Including additional variables in the calculations makes it possible to control for some confounding variables when measurements are available.

We recommend that users standardize pairs to meet statistical requirements of subsequent statistical tests (i.e., equal variances among pairs). Standardization has not typically been implemented for pairwise comparisons, but it is necessary if one wishes to use parametric statistical tests that make assumptions about homoscedasticity. When contrasts are standardized, distantly related pairs are selected less often. This may be useful if large differences are informative only when the species are closely related (e.g., to control for possibly unknown confounding variables) or if comparisons should be made between closely related species (e.g., because of biological differences that limit comparability of experimental results). Standardization as such affects the selection of pairs.

Another argument for standardization is that fewer traits should change on shorter branches, and thus it helps to control for confounding variables. However, standardization may exaggerate evolutionary differences for close relatives when differences are due to sampling error or within-species variation (Purvis and Webster 1999). It can therefore overestimate the importance of certain species pairs if they are close relatives. We may sometimes expect a larger absolute change in some trait, regardless of its rate of change, to be more valuable in testing a hypothesis than a small change over a short branch. For example, brain size that increases by an order of magnitude might be a stronger test than a smaller amount of brain change, even if the small change occurs over a small branch. Using the program that we provide, the choice of standardization is left up to the user (with the default option to standardize scores) and is based on his or her preferences, the assumptions of subsequent methods, and the particulars of the biological system.
Phylogenetic targeting works best for continuous traits, but it can also be used with discrete traits. However, phylogenetic targeting that is based purely on discrete characters is more challenging because the number of distinct differences is typically smaller. In such cases, it is common to find that numerous pairs have the maximal possible score. This will ultimately result in multiple optimal solutions in the maximal pairing algorithm. However, because the current implementation returns only one optimal solution, it is difficult to evaluate its uniqueness. Possible work-arounds would be to add a continuous variable or to standardize contrasts; both of these would help to generate variation in the scores and thus aid in deciding among the possible pairs of taxa.

The maximal pairing algorithm falls in a class of general combinatorial optimization problems that are of considerable interest in comparative phylogenetics and, more generally, bioinformatics. Several modifications of this algorithm have practical importance as well. For example, the algorithm could be modified to select only a fixed number of pairs (given by the researcher), thereby incorporating the fact that limited resources are available to select species for future study. This important variant is described in more detail elsewhere (see Arnold and Stadler 2010). It might also be desirable to take into account the conservation statuses of different species to ensure that species are studied before they become extinct. More generally, the selection of species could be based not solely on pairwise comparisons but on the full set of contrasts, possibly in combination with examining the raw data space or regularly sampling character values along the entire range of a character of interest. Here we laid down the foundation for systematically identifying species for future study. Many possible extensions and modifications of the approach are possible, particularly as they relate to alternative ways of sampling species.

In summary, we provided a systematic method of selecting species for future study that offers greater statistical power to test adaptive hypotheses, as compared with a random selection of species. With this method of phylogenetic targeting, it is also possible to control for confounding variables, incorporate alternative hypotheses, and make use of existing data on the trait of interest. It therefore provides a way to guide the selection of species relative to a priori hypotheses. Through our Web-based computer program, other researchers are able to easily implement this approach in a flexible and user-friendly way.

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Literature Cited


