Phylogenies, the Comparative Method and Parasite Evolutionary Ecology

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ABSTRACT

A growing number of comparative analyses in the field of parasite evolution and ecology have used phylogenetically based comparative methods. However, the comparative approach has not been used much by parasitologists. We present the rationale for the use of phylogenetic information in comparative studies, and we illustrate the use of several phylogenetically based comparative methods with case studies in parasite evolutionary ecology. The independent contrasts method is the most popular one, but presents some problems for studying co-adaptation between host and parasite life traits. The eigenvector method has been recently proposed as a new method to estimate and correct for phylogenetic inertia. We illustrate this method with an investigation of patterns of helminth parasite species richness across mammalian host species. This method seems to perform well in situations where host and parasite phylogenies are not perfectly congruent, but one might still want to correct for the effects of both. Finally, we present a method recently proposed for variation partitioning in a phylogenetic context, i.e. the phylogenetically structured environmental variation.

1. INTRODUCTION

One of the most powerful approaches to the study of adaptation and evolution in general involves comparing different species and searching for a predictable and consistent fit between their traits and some environmental variable of interest. This is the essence of the comparative method (Harvey and Pagel, 1991). It has now become almost globally accepted that one needs to take into account phylogenetic information in cross-species studies of adaptation (Felsenstein, 1985). The basic argument is mostly a statistical one, with phylogenetically closely related species not representing truly independent samples. Simulation studies and empirical analyses have shown that ignoring phylogenetic relationships among species included in a comparative analysis may lead to spurious conclusions due to high type I or type II errors (Gittleman and Luh, 1992; Purvis *et al*., 1994; Diaz-Uriarte and Garland, 1996). A growing number of comparative analyses in the field of parasite evolution and ecology have used phylogenetically based comparative methods (Poulin, 1995, 1998; Morand, 2000), but their use is far from universal. Here, we present the rationale for the use of phylogenetic information in comparative studies, and we illustrate the use of several phylogenetically based comparative methods with case studies in parasite evolutionary ecology.

2. PHYLOGENETIC EFFECTS AND CONSTRAINTS, AND THE NEED FOR PHYLOGENIES

Several terms have been proposed in the literature to refer to a potential influence of evolutionary history, i.e. phylogeny, on the observed pattern of diversity: phylogenetic effects, phylogenetic constraints, phylogenetic niche conservatism or phylogenetic inertia.

Derrickson and Ricklefs (1988) placed much emphasis on the difference between phylogenetic effects and phylogenetic constraints. A phylogenetic effect is only the expression of the tendency of related species to be similar because they share a common history, whereas a phylogenetic constraint is the effect of history on the changes in diversification of a given clade (Derrickson and Ricklefs, 1988). In this sense phylogenetic effects and phylogenetic inertia are equivalent. However, according to McKitrick (1993), the definition of phylogenetic constraints, rather than placing the emphasis on the causes themselves, focuses more on the consequences of the constraints. McKitrick (1993) proposed the following definition in which a phylogenetic constraint is ''any result or component of the phylogenetic history of a lineage that prevents an anticipated course of evolution in that lineage''. This definition is close to the definition of the term exaptation proposed by Gould and Vrba (1982). The influence of an ancestor on its descendants has been termed phylogenetic inertia by Harvey and Pagel (1991). Finally, the concept of phylogenetic niche conservatism refers to shared attributes of phylogenetic inertia and ecological factors (Grafen, 1989; Harvey and Pagel, 1991).

So why are all these concepts so important for comparative analyses in parasite evolutionary ecology? Consider as an example the evolution of body sizes in parasitic nematodes. There is a wide range of adult body sizes among extant species of parasitic nematodes, from a couple of millimetres to several centimetres in length. Body size is the most influential determinant of parasite fecundity and therefore fitness (Skorping *et al.*, 1991; Morand 1996), and investigating what determines its evolution is thus extremely relevant. The existence of phylogenetic effects becomes apparent when one looks at the distribution of body sizes among higher taxa of nematodes. For instance, lets consider parasitic nematodes belonging to the families Oxyuridae and Ascarididae, which are common intestinal parasites of mammals. Whatever the host species in which they occur, members of the family Ascarididae are almost invariably larger-bodied than members of the family Oxyuridae (Anderson, 2000). Clearly, oxyurids must have inherited their small size from their common ancestor, whereas ascaridids have inherited their large size from their common ancestor. Species within a family are not independent of one another because they share traits simply

by being related. It is essential to take phylogenetic relatedness into account in a comparative analysis of the evolution of body size in parasitic nematodes. This is true of practically all traits, in any taxon, whether parasitic or not. The additional problem that is mainly restricted to parasites and other obligate symbionts is that their traits are not only inherited from their ancestors, but their expression is also likely to be an adaptation to their environment, i.e. their hosts. Hosts have their own evolutionary history, i.e. their own phylogeny. Getting back to nematode body sizes, there is good evidence showing that adult body sizes covary with host sizes (Morand *et al*., 1996); ignoring how host sizes themselves have evolved could lead to erroneous conclusions. Some host groups, such as bats and rodents among mammals, have similar body sizes despite having completely different phylogenetic origins, whereas more closely-related host species (e.g. among rodents, from tiny mice to the 50 kg capybara) may differ widely in body size despite a more recent phylogenetic divergence. So, in investigations of traits such as parasite body sizes, the ideal scenario might involve an analysis that takes into account *both* the phylogenies of hosts and parasites. Clearly, ignoring phylogeny altogether should not be an option anymore.

The major problem with comparative analyses of parasites has been that most of them are small and cryptic, without fossil records (except in rare exceptions), and their diversity has received much less attention than that of free-living animals (Poulin and Morand, 2000). For a long time, robust phylogenetic hypotheses were lacking for most groups, which limited the application of comparative approaches to studies of parasite ecology and evolution. However, recent developments in molecular phylogenetics have provided numerous historical frameworks that allow the investigation of parasite evolution in a proper phylogenetic context (e.g., Blaxter *et al*., 1998; Littlewood and Bray, 2001).

3. THE PHYLOGENETICALLY INDEPENDENT CONTRASTS METHOD

The phylogenetically independent contrasts method (Felsenstein, 1985; Martins and Garland, 1991; Garland *et al*., 1992) has been developed to resolve the problem of non-independence of data (i.e., traits measured across different species) in comparative studies. Felsenstein (1985) suggested a procedure for calculating comparisons between pairs of taxa at each bifurcation in a known phylogeny; since these bifurcations represent independent evolutionary events, contrasts between sister taxa issued from one bifurcation are thus independent from contrasts computed for other

bifurcations. Since its development in 1985, the independent contrasts method has become the most widely used in comparative biology, even if sister group analyses can also be performed.

In a phylogenetic tree, the independent events (on which an analysis can be performed) correspond to ancestral (or internal) nodes that give rise to daughter branches. For each internal node, values for a given variable are obtained by averaging the values of its own daughter branches. Then the difference for each variable between the two daughter branches of each node is calculated. In the calculation of contrasts, the direction of subtraction is arbitrary. Multiple nodes (i.e. unresolved polytomies) can be treated in a way that gives a single contrast (Purvis and Garland, 1993). Pairs of sister branches that diverged a long time ago are likely to produce greater contrasts than pairs of sister branches that diverged recently. It is thus necessary to standardise each contrast through division by its standard deviation where the standard deviation of a contrast is the square root of the sum of the branch lengths issued from it (Garland *et al*., 1992). The main assumption of independent contrasts is a Brownian model of character evolution or random walk model. Changes in the mean phenotype are expected to occur at a constant rate and to be non-directional. The Brownian model of character evolution can be tested by regressing the absolute values of contrasts against the estimated nodal values. In the absence of information on branch length, one can assume each branch length to be equal to unity. Another method is proposed by Grafen (1989) for assigning arbitrary lengths. In this method the age of a node is assigned as the number of daughter groups descended from that node minus one. Nevertheless, Garland *et al*. (1992) showed that using arbitrary or real branch lengths often leads to similar results. In order to check that contrasts are properly standardised it is suggested to perform a regression of the absolute values of standardised contrasts versus their standard deviations. In case of positive relationship it is necessary to transform branch lengths before computing standard deviations (Garland *et al*., 1992). Contrasts can then be analysed using standard parametric tests, although all correlations between contrasts are forced through the origin. Non-parametric methods can also be used to analyse the contrasts (e.g. sign test). Several programs have been developed for performing independent contrasts analyses, e.g. CAIC (Purvis and Rambaut, 1995). Clearly, not taking into account the phylogenetic information may lead to spurious results, as illustrated by Morand and Poulin (1998) for the relationship between mammalian population density and parasite species richness. A non-phylogenetic approach (cross-species comparisons) leads to the conclusion that parasite species richness correlates negatively with mammalian population density (Figure 1A), whereas the use of the independent contrasts method showed a positive relationship (Figure 1B).

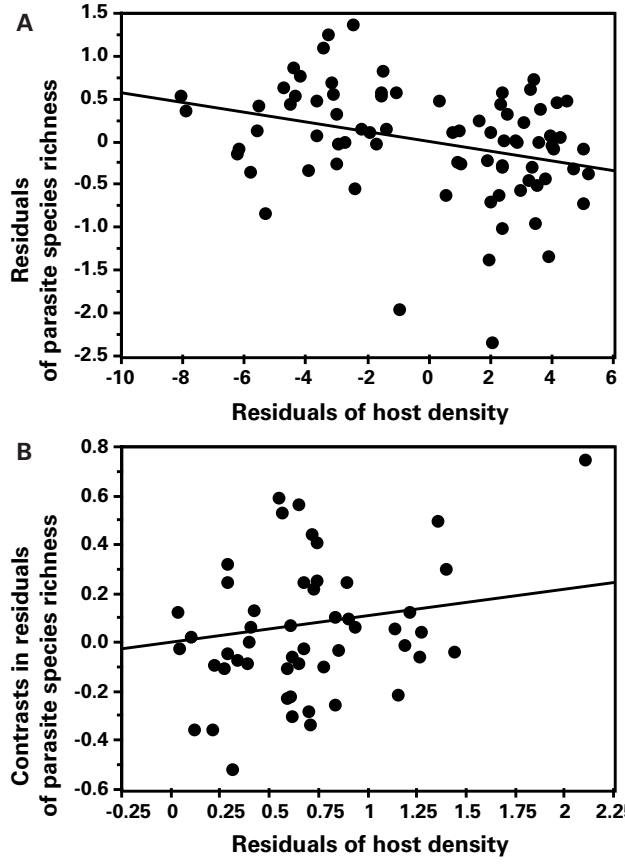

Figure 1 Relationship between host body density and parasite species richness (both variables controlled for host sampling effort) (A) using cross-species comparisons ($p < 0.01$), (B) using independent contrasts ($p < 0.05$) (redrawn after Morand and Poulin, 1998).

A robust and accurate phylogeny is the most important assumption of the independent contrasts method (Harvey and Pagel, 1991; Symonds, 2002) as well as for sister group analyses. Because of the general lack of phylogenetic information, the use of randomly generated phylogenetic trees has been proposed as an alternative (Losos, 1994; Martins, 1996; Abouheif, 1998). For instance, this procedure was used by Simkova *et al.* (2000) for the investigation of morphological adaptations of *Dactylogyrus* spp. to their Cyprinid hosts. However, a recent simulation study performed by

Symonds (2002) showed that random phylogenies may actually perform worse than analyses using raw data with no attempt to control for phylogeny.

With respect to comparative analysis of parasites, the problem is how to use the independent contrasts method when investigating the coadaptation of traits in hosts and their parasites, since ideally phylogenetic information from both hosts and parasites should be taken into account simultaneously. The independent contrasts method does not allow for this requirement. However, if the trait studied is clearly influenced much more by the phylogenetic history of parasites rather than hosts, or vice versa, then the independent contrasts method is a powerful tool for studies on parasites.

4. DIVERSITY AND DIVERSIFICATION

Instead of continuous traits, one may want to investigate differences in rates of diversification among related taxa. The study of biodiversity involves the measurement of net rates of diversification (rates of speciation minus rates of extinction). Extinction and speciation rates may differ among clades, and they may explain the relative "proliferation" of species in one clade compared to others. The search for causal links between key innovations and the diversification of platyhelminths (Brooks and McLennan, 1991) has stimulated several investigators to study diversification in parasite lineages.

Diversification rates can be tested using sister group analyses (Barraclough *et al*., 1999). Desdevises *et al*. (2001) tested whether the level of host specificity affects parasite species diversification within a monogenean family. They were the first to use the MacroCAIC program (Agapow and Isaac, 2002), derived from CAIC (Purvis and Rambaut, 1995), in a study on parasites. MacroCAIC is designed specifically for studies of diversification, and uses a basic approach based on phylogenetically independent contrasts. Desdevises *et al*. (2001) found no effect of host specificity on the species diversification of the group of monogeneans they investigated. Here, we investigate the effect of body size on monogenean diversification across all monogenean families using the data collected by Poulin (2002). Body size is often believed to be a key driver of diversification rates in animals in general (Orme *et al*. 2002a,b), and our example illustrates how this fundamental issue can be addressed with parasites in a phylogenetic context.

MacroCAIC allows one to use species richness as a variable in a comparative analysis to estimate whether other traits (here body size) are associated with high speciation rate (species richness, genus richness, or

family richness within a higher clade). Species, genus, or family richness per clade cannot be used as any other continuous variable with independent contrasts because, in this case, the estimated richness value at each internal node in the phylogeny is not the average of the values of branches issued from the node, but their sum.

In our example using monogenean body size and diversification, the variables tested were:

- the natural log of the CLS/CHS ratio; where CLS is the species richness of the clade with lower mean body size, for each node of the phylogeny, and CHS is the species richness of its sister clade with larger mean body size;
- the natural log of the CLG/CHG ratio; where CLS is the number of genera in the clade with lower mean body size, for each node of the phylogeny, and CHS is the number of genera in its sister clade with larger mean body size;
- the natural log of the CLF/CHF ratio; where CLS is the number of families in the clade with lower mean body size, for each node of the phylogeny, and CHS is the number of families in its sister clade with larger mean body size.

When the ratio is 1, i.e., ln ratio = 0, the number of species (or the number of genera or families) in each sister clade is the same, and when the ratio is greater than one (positive ln ratio), the clade with the most species (or genera or families) is also the one with the highest mean body size. The analyses were then performed across pairs of sister clades at each internal node of the phylogeny.

The ln(CLS/CHS), the ln(CLG/CHG) and the ln(CLF/CHF) were regressed against standardised contrasts for ln(mean body size), using MacroCAIC. If the hypotheses tested are true, we should observe an increase of diversification with mean body size.

The regression equations using contrasts (forced through origin) were:

$\ln(\text{CLS/CHS}) = -1.14 \ln(\text{mean body size})$ ($R^2 = 0.023$, $p = 0.35$, Figure 2A).
$\ln(\text{CLG/CHG}) = -0.50 \ln(\text{mean body size})$ ($R^2 = 0.007$, $p = 0.62$, Figure 2B).
$\ln(\text{CLF/CHF}) = -1.62 \ln(\text{mean body size})$ ($R^2 = 0.13$, $p = 0.10$, Figure 2C).

Thus, no significant relationships were found between mean body size and diversification at any taxonomic level among the Monogenea, i.e. clades with higher species richness are not characterized by smaller or larger mean body size than their sister clades. Nevertheless, the methods are now available, and similar comparative studies of parasite diversification are now possible within other parasite groups with well-resolved phylogenies.

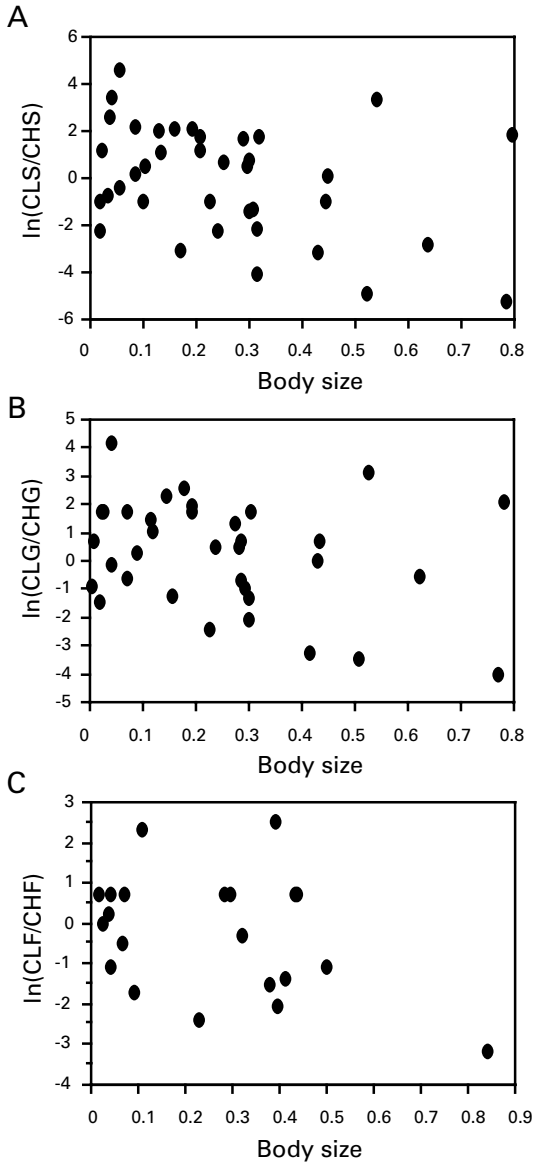

Figure 2 Relationships between the diversification ratio between sister clades of monogeneans (clade with high diversification/clade with low diversification) and mean body size (in ln), computed on independent contrasts using MacroCAIC. The analyses were performed at the level of (A) species, (B) genera and (C) families. None of the relationships were significant.

5. THE PHYLOGENETIC EIGENVECTOR METHOD

The phylogenetic eigenvector regression (PER) has been recently proposed by Diniz-Filho *et al*. (1998) as a new method to estimate and correct for phylogenetic inertia, based on an approach that is very different from that used when computing phylogenetically independent contrasts.

Diniz-Filho *et al*. (1998) only used the first principal coordinates selected with reference to a broken-stick model. All the principal coordinates extracted from the distance matrix that are significantly related to the dependent variable(s) can be used. The broken-stick model does not provide reasons to select variables of importance for the explanation of the dependent variable. As distances used might not always be Euclidean, negative eigenvalues may be produced (see Legendre and Legendre, 1998). In this case, it is possible to apply some correction methods, as presented in Gower and Legendre (1986) or Legendre and Legendre (1998). Again, a phylogenetic distance matrix can be obtained either from the raw data (e.g. sequence alignments), which avoids the reconstruction of a tree, or computed from a patristic distance matrix representing a phylogenetic tree. In simple terms, this method consists of transforming phylogenetic information into numbers that can then be used as any other variable.

A principal coordinate analysis (PCoA) is then performed on a pairwise phylogenetic distance matrix between species. Eigenvectors and eigenvalues are extracted from this analysis (Figure 3). Traits under analysis are regressed on eigenvectors retained by a broken-stick model and the residuals express the independent evolution of each species, whereas estimated values express phylogenetic trends in the data. Multiple regression analyses can also be performed using eigenvectors as predictors (see below).

5.1. Example Using Mammals

We illustrate this method with an investigation of patterns of helminth parasite species richness across mammalian host species. We used data on 79 mammal species (see Morand and Poulin (1998) for sources). Data on various life history traits were obtained from the literature (see Morand (2000) for sources). We also used recent advances in the knowledge of mammalian phylogenetic relationships (Cooper and Fortey, 1998; Murphy *et al*., 2001) to derive a working phylogeny of the 79 species included in the analyses. In this case, we correct for host phylogeny, as we expect host features to influence the number of parasite species they harbour.

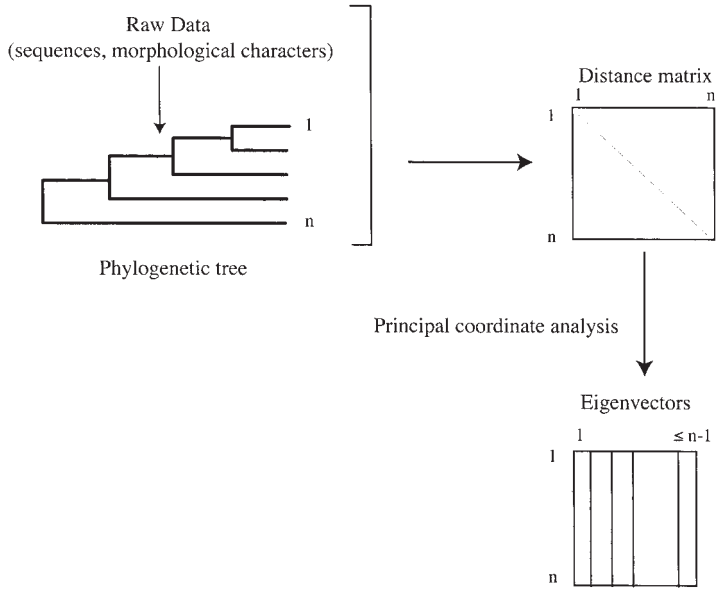

Figure 3 Principal coordinate analysis (PcoA) performed on phylogenetic distance matrix in order to extract eigenvectors representing the phylogenetic information (the maximum number of eigenvectors is $(n-1)$ but this may not always be the case) (after Desdevises, 2001).

Morand and Harvey (2000), using the independent contrasts method, have found that parasite species richness is positively correlated with the basic metabolic rate of mammal hosts and negatively correlated with mammal longevity. Basal metabolic rate (BMR) gives an indication of energy expenditure by animals, and represents the minimum energetic cost necessary for maintaining the activity of an organism. BMR, which scales with body mass, has been related to a great number of variables such as body mass, dietary habits, brain size, and reproductive strategies, and the reasons for the residual variation have been discussed (see Morand and Harvey, 2000).

We applied the PER to these data and all partial regression coefficients of the linear correlations are given in Figure 4. The subset of determinants was obtained using a stepwise regression with a backward elimination procedure.

First, we detected a phylogenetic effect on many of the host life history traits as depicted by linear correlations between these traits and the first principal coordinate of the phylogenetic matrix. It appears that the observed parasite species richness is not correlated with any of the principal eigenvectors. Second, the stepwise procedure allowed the selection of three

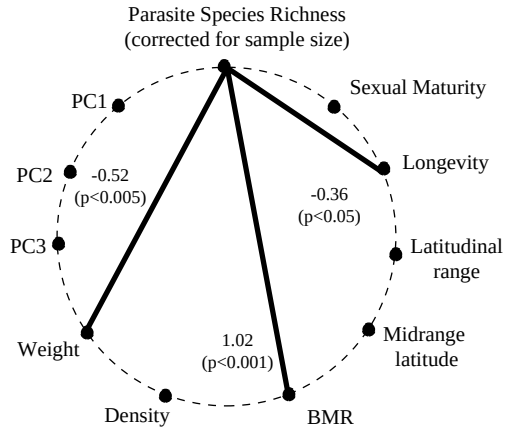

Figure 4 Diagram of the correlations between parasite species richness (corrected for host sampling size), host attributes (sexual maturity, longevity, weight, density, geographic distribution) and eigenvectors representing host phylogeny (principal coordinates: PC1, PC2, PC3), and results of a backward procedure on a multiple linear regression to select a subset of explanatory variables (determinants) of parasite species richness of mammals.

determinants of parasite species richness (corrected for sampling size), namely host body weight, host BMR and host longevity. We found a negative effect of body size and much more importantly a negative effect of host longevity ($R^2 = 0.58$, $p = 0.0011$). These results illustrate how the PER can be used in multivariate analyses including corrections for phylogenetic effects.

6. THE STUDY OF HOST–PARASITE CO-ADAPTATION USING THE INDEPENDENT CONTRASTS METHOD

Although the phylogenetically independent contrasts method does not allow for the simultaneous inclusion of two phylogenies, such as that of hosts and parasites, it can be used to control for both phylogenies in the unusual case where both phylogenies are perfectly congruent. In other words, in such cases one needs only to derive contrasts from one phylogeny to correct for the other one at the same time. This is particularly important when comparing the effect of one host trait (which may constrained by host phylogeny) on one parasite trait (which may constrained by parasite phylogeny).

Morand *et al.* (2000) examined the relationship between body size of pocket gophers and body size of their chewing lice (Hafner *et al.*, 1994; Hafner and Page, 1995). Because chewing lice of mammals grasp the hair of the host in a semi-circular head groove, the head groove appears to be critically important to the louse's survival. Morand *et al.* (2000) hypothesised that the observed correlation between louse body size and gopher body size (Harvey and Keymer, 1991) may reflect a relationship between gopher hair-shaft diameter and louse head-groove dimensions, suggesting that there is a "lock-and-key" relationship between these two anatomical features.

They used the CAIC program to obtain independent contrast values for gopher and louse body sizes (Purvis and Rambaut, 1995). However, because the independent contrasts method compares the nodes of a single phylogeny, it was necessary that the host and parasite phylogenies being compared be topologically identical. Accordingly, Morand *et al.* (2000) restricted the analysis to the congruent portions of the gopher and louse phylogenies. They showed a positive relationship between gopher hair-shaft diameter and louse head-groove width and concluded that changes in body size of chewing lice may be driven by a mechanical relationship between the parasite's head-groove dimension and the diameter of the hairs of its host. Louse species living on larger host species may be larger simply because their hosts have thicker hairs, which requires that the lice have a wider head groove. This study of gopher hair-shaft diameter and louse head-groove dimensions suggests that there is a "lock-and-key" relationship between these two anatomical features. It also illustrates how the phylogenetically independent contrasts method can, in some cases, simultaneously control for *both* host and parasite phylogenies.

7. THE STUDY OF HOST–PARASITE CO-ADAPTATION USING PER

In most situations, however, host and parasite phylogenies are not perfectly congruent, but one might still want to correct for the effects of both. In these cases, PER provides a solution.

We investigate the covariation of life history traits between hosts and parasites. We reinvestigate the case of primates and their oxyurid parasites (Sorci *et al.*, 1997). Sorci *et al.* (1997) tested the hypothesis of a positive covariation between parasite body length (reflecting parasite longevity) and host longevity using the independent contrasts method. However, this method can only be applied when there is a high degree of cospeciation in

the host system; otherwise, it ignores the phylogenetic information of either hosts or parasites. In the present example, the phylogenies of primates and their oxyurid nematodes are not perfectly congruent (Figure 5), and it would be preferable to control for the influence of both phylogenies.

The use of eigenvalues allows this to be done. Two principal coordinate analyses (PCoA) are first performed on a pairwise phylogenetic distance matrix between species of primates and between species of oxyurid parasites (Figure 4). Eigenvectors and eigenvalues are extracted from this analysis.

We then regress life history traits on eigenvectors retained by a broken-stick model, which retains the statistically explaining eigenvectors (see Legendre and Legendre, 1998) independently for primates and for oxyurid parasites. All traits are then corrected for *all* phylogenetically confounding effects and then can be compared.

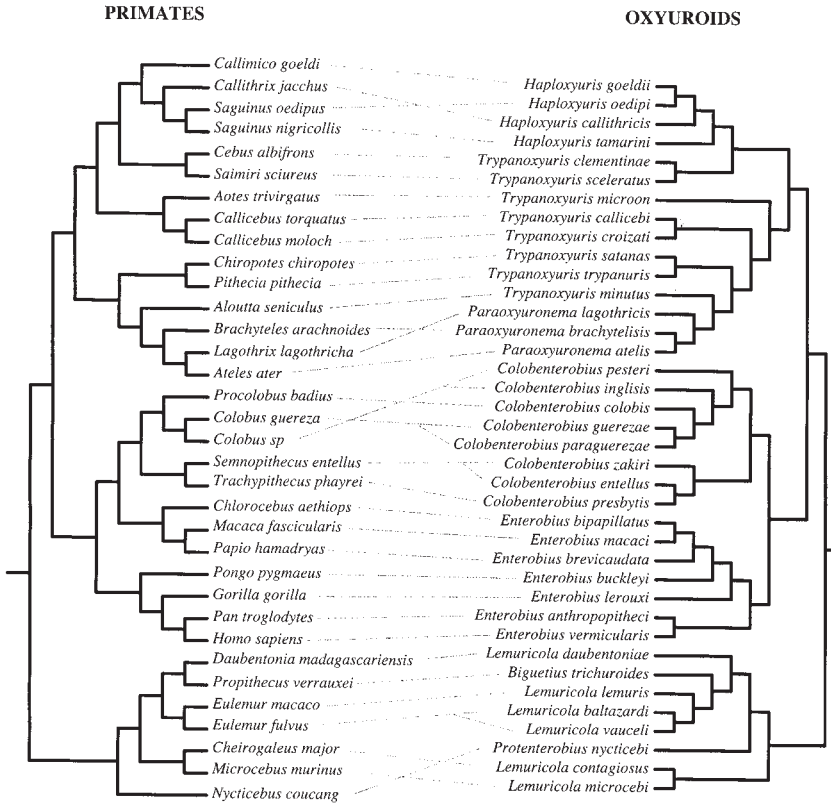

Figure 5 Tanglegram of phylogenies of oxyuroid nematodes and their primate hosts (after Hugot, 1999).

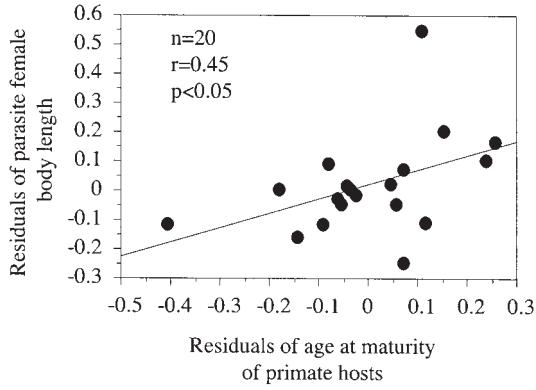

Figure 6 Relationship between female body length of oxyuroids and primate longevity (corrected for body weight). Both variables are controlled for phylogenetic confounding effects using PER (phylogenetically eigenvector regression) (see text).

We find that female parasite body length is positively correlated with host longevity (Figure 6) after correcting for host body mass and phylogeny using PER. This result confirms the previous finding of Sorci *et al.* (1997). However, the great advantages of this method are that it corrects for both host and parasite phylogenies and that it can be used in host–parasite system where the degree of cospeciation is very low (Desdevises *et al.*, 2002a, 2002b) and even if the degree of host specificity is low.

8. SCEPTICISM ABOUT COMPARATIVE METHODS: WHY BOTHER WITH PHYLOGENY?

Several authors have questioned the use of phylogenetic comparative method (Ricklefs and Starck, 1996; Björklund, 1997; Price, 1997). Leroi *et al.* (1994) argued that comparative methods are "valuable for examining the evolutionary history of traits but they will often mislead in the study of adaptive processes". Their major concern was that we know very little about the evolutionary genetic mechanisms responsible for the distribution of traits among species. They claimed that it is very difficult to justify any evolutionary scenario without evidence of historical selection forces and, more important, the genetic relations among traits. Some of their arguments concern mainly the invocation of constraints in the explanation of either adaptation or phylogenetic conservatism. Their second criticism is about "the confounding of the causal influence of selection with that of genetic

correlations''. This is a more serious critique but, again, the problem applies more to inferences about the causality of the correlations than to the methods themselves. Indeed, Leroi *et al.* (1994) concluded their essay with the acknowledgement ''that the methods of comparative biology and genetics might be usefully combined''.

A different kind of criticism came from Westoby *et al.* (1995a, b). Their concern was that a phylogenetic correction (i.e. phylogenetically based comparative analysis) is not a correction, but rather a conceptual decision that gives priority to one interpretation over another. The comparative method, and particularly the independent contrasts method, assumes that part of the variation of a given trait is correlated with phylogeny and another part is correlated with ecology. They criticised that the procedure first removes the phylogenetic influences before estimating the influence of present-day ecological factors.

The problem is that we only know the actual phenotypes of species under the current selective regimes. We do not know what may have happened to ancestral species and what their phenotypes were. Hence, phylogenetic conservatism may be described as follows: ''the ancestor of a lineage possesses a constellation of traits, enabling it to succeed in a particular habitat and disturbance regime, through a particular life history and physiology. The lineage will therefore leave most descendants in similar niches. This niche conservatism in turn will tend to sustain a similar constellation of traits in descendants of the lineage'' (Westoby *et al.*, 1995a).

Harvey *et al.* (1995) tried to provide an answer to this criticism by emphasising that the independent contrasts method does not remove phylogenetic effects, but produces plots in which all the variation of the data set in one variable is graphed against all the variation in the other variable. In this way, phylogenetic niche conservatism means that adaptations to different components of the niche will be correlated (Harvey *et al.*, 1995). In any event, perhaps the best resolution of this debate would be to use methods that allow both the variance due to phylogenetic influences and that due to present ecological causes to be evaluated; we now turn to one such method.

9. PHYLOGENETICALLY STRUCTURED ENVIRONMENTAL VARIATION

Desdevises *et al.* (2001 (unpublished) and 2002a) have re-examined the controversy initiated by Westoby *et al.* (1995a) (but see also Ackerly and Donoghue, 1995; Fitter, 1995; Harvey *et al.*, 1995; Rees, 1995; Westoby

et al., 1995b, c). Westoby *et al.* (1995a) pointed out that the phylogenetic portion of the total variance may contain a phylogenetic effect related to ecology, which Harvey and Pagel (1991) called "phylogenetic niche conservatism". This includes the shared attributes that related species have acquired because they tend to occupy similar niches during their evolutionary history. Westoby *et al.* (1995) proposed to partition the variance of the data set in three components (Figure 1 from Westoby *et al.*, 1995a; here Figure 7, equivalent to fraction [a], [b] and [c]): a part strictly due to ecology ([a]), a part strictly due to phylogeny ([c]), and a part due to the common influence of these two factors ([b]), which was called "phylogenetically-structured environmental variation", an expression equivalent to "phylogenetic niche conservatism" as used by Desdevises (2001) and Desdevises *et al.* (2002a).

Desdevises *et al.* (2002a) proposed a method for variation partitioning in a phylogenetic context, which was already proposed by Borcard *et al.* (1992) and Borcard and Legendre (1994) for the analysis of spatially structured ecological data sets.

In a phylogenetic context, we express phylogeny as a distance matrix following PER previously discussed. The phylogeny is expressed in the form of principal coordinates computed from a patristic distance matrix, following Diniz-Filho *et al.* (1998).

The method used to partition the variance is as follows. Let Y be the dependent variable (i.e. parasite species richness or PSR), X_E the ecological explanatory variable(s) (i.e. BMR, host weight, host longevity), and PCs the principal coordinates representing the phylogeny.

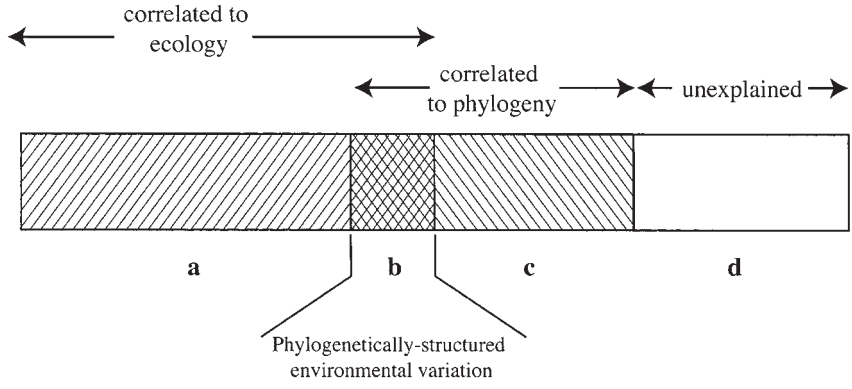

Figure 7 Partitioning the variance of the data, with a part strictly due to ecology ([a]), a part strictly due to phylogeny ([c]), a part due to the common influence of these two factors ([b]), and the unexplained variance (d) (after Desdevises *et al.*, 2002).

Table 1 Some software packages available for comparative and statistical analyses.

Software	Website
ACAP	http://www.stanford.edu/~dackerly/ACAP.html
ANCML	http://www.zoology.ubc.ca/~schluter/ancml.html
CAIC	http://www.bio.ic.ac.uk/evolve/software/caic/
MacroCAIC	http://www.bio.ic.ac.uk/evolve/software/macrocaic/
COMPARE	http://compare.bio.indiana.edu/
DISCRETE	http://www.ams.rdg.ac.uk/zoology/pagel/mppubs.html
PDAP	http://cnas.ucr.edu/~bio/faculty/Garland/PDAP.html
TFSI	http://life.bio.sunysb.edu/ee/ehab/
R, Permute!, ParaFit	http://www.fas.umontreal.ca/biol/casgrain/en/siteOutline.html

We first compute a regression of Y on X_E. The coefficient of multiple determination of the regression, R^2, is equal to the fraction [a + b] of the variance decomposition (a stepwise procedure can select a subset of explanatory variables). Second, we compute a multiple regression of Y on PCs. R^2 in this case is equal to [b + c]. Third, we compute a multiple regression of Y on both X_E and PCs. R^2 is now equal to [a + b + c].

We can find [d] = 1 − [a + b + c], which is the unexplained variance (note [a] can be found from [a + b + c] − [b + c]).

This method is here illustrated with an application to the same data set on mammal hosts and the species richness of their helminth parasites that we used earlier.

We find [a + b] = 0.581 ($p < 0.0001$) with host BMR, weight and longevity as explanatory variables of PSR.

We find [b + c] = 0.169 ($p = 0.008$) and [a + b + c] = 0.711 ($p < 0.0001$)

This allows us to estimate the phylogenetically structured environmental variation [b] = 0.039, i.e., about 4% of the variance in parasite species richness among mammal species is due to phylogenetically structured environmental variation. The phylogenetic inertia is given by [c] = 0.13, i.e., 13% of the variance in parasite species richness among mammal species.

This result can mean that hosts with comparable PSR tend to occupy the same kind of ecological niche, but this trend is very weak because the phylogenetic inertia [b] is low; also, because of the very low value of phylogenetically structured environmental variation [c], phylogenetic and ecological influences on PSR are almost independent.

This method of partitioning the variance between phylogenetic and ecological causes not only serves to silence the critics of phylogenetically

based comparative approaches, but it allows one to quantify precisely the importance of phylogeny for any character.

10. CONCLUSIONS

Data on a wide range of biological features of parasites have been accumulating steadily over the past several years. This information may contain the key to understanding how parasites evolve in response to selective pressures from their hosts and the external environment. Modern comparative methods that incorporate phylogenetic information allow us to make full use of these data. They allow several questions to be answered. For instance, how are two traits correlated with each other independently of the phylogenetic relationships of their bearer? What portion of the variation in one trait is due to ecological influences as opposed to historical or phylogenetic effects? Here, we briefly reviewed some of the current comparative methods available to address these and related questions, providing references where readers can find out more about these methods. As pointed out recently (Poulin, 1998), the comparative approach has not been used much by parasitologists. Our hope is that this essay will stimulate more researchers to adopt these methods and apply them to the study of parasite evolutionary ecology.

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REFERENCES

Abouheif, E. (1998). Random trees and the comparative method: a cautionary tale. *Evolution* **52**, 1197–1204.

Ackerly, D.D. and Donoghue, M.J. (1995). Phylogeny and ecology reconsidered. *Journal of Ecology* **83**, 730–732.

Agapow, P.-M. and Isaac, N.J.B. (2002). MacroCAIC: revealing correlates of species richness by comparative analysis. *Diversity and Distributions* **8**, 41–43.

Anderson, R.C. (2000). *Nematode parasites of vertebrates: Their Development and Transmission*. Wallingford: CABI Publishing.

Barraclough, T.G., Hogan, J. and Vogler, A.P. (1999). Testing whether ecological factors promote cladogenesis in a group of tiger beetles (Coleoptera: Cicindelidae). *Proceedings Royal Society London B* **266**, 1061–1067.

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Doriis, M., Frisse, L.M., Vida, J.T. and Thomas, W.K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**, 71–75.

Björklund, M. (1997). Are 'comparative methods' always necessary? *Oikos* **80**, 607–612.

Borcard, D. and Legendre, P. (1994). Environmental control and spatial structure in ecological communities: an example using oribatid mites (Acari, Oribatei). *Environmental and Ecological Statistics* **1**, 37–53.

Borcard, D., Legendre, P. and Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology* **73**, 1045–1055.

Brooks, D.R. and McLennan, D.A. (1991). *Phylogeny*, *Ecology*, *and Behavior*. *A Research Program in Comparative Biology*. Chicago, The University of Chicago Press.

Cooper, A. and Fortey, R. (1998). Evolutionary explosions and the phylogenetic fuse. *Trends in Ecology and Evolution* **13**, 151–156.

Derrickson, E.M. and Ricklefs, R.E. (1988). Taxon-dependent diversification of life history traits and the perception of phylogenetic constraints. *Functional Ecology* **2**, 417–423.

Desdevises, Y. (2001). Recherche des déterminants de la spécificité parasitaire dans le modèle *Lamellodiscus* (Diplectanidae, Monogenea)-Sparidae (Teleostei) en Méditerranée. Unpublished PhD thesis, Université de Perpignan, 317 pp.

Desdevises Y., Morand, S. and Oliver, G. (2001). Linking specialisation to diversification in the Diplectanidae Bychowsky 1957 (Monogenea, Platyhelminthes). *Parasitology Research* **87**, 223–230.

Desdevises, Y., Legendre, P. and Morand, S. (2002a). Evolution and determinants of host specificity in the genus *Lamellodiscus* (Monogenea). *Biological Journal of the Linnean Society* **77**, 431–443.

Desdevises, Y., Morand, S., Jousson, O. and Legendre, P. (2002b). Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): the study of a complex host-parasite system. *Evolution* **56**, 2459–2471.

Diaz-Uriarte, R. and Garland, T. Jr. (1996). Testing hypotheses of correlated evolution using phylogenetically independent contrasts: sensitivity to deviations from brownian motion. *Systematic Biology* **45**, 27–47.

Diniz-Filho, J.A.F., de Sant'Ana, C.E.R. and Bini, L.M. (1998). An eigenvector method for estimating phylogenetic inertia. *Evolution* **52**, 1247–1262.

Felsenstein, J. (1985). Phylogenies and the comparative method. *American Naturalist* **125**, 1–15.

Fitter, A.H. (1995). Interpreting quantitative and qualitative characteristics in comparative analyses. *Journal of Ecology* **83**, 730.

Garland, T. Jr., Harvey, P.H. and Ives, A.R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *American Naturalist* **41**, 18–32.

Gittleman, J.L. and Luh, H.K. (1992). On comparing comparative methods. *Annual Review of Ecology and Sytematics* **23**, 383–404.

Gould, S.J. and Vrba, E.S. (1982). Exaptation: a missing term in the science of form. *Paleobiology* **8**, 4–15.

Gower, J.C. and Legendre, P. (1986). Metric and Euclidean properties of dissimilarity coefficients. *Journal of Classification* **3**, 5–48.
Grafen, A. (1989). The phylogenetic regression. *Philosophical Transactions of the Royal Society of London B* **326**, 119–157.
Hafner, M.S. and Page, R.D.M. (1995). Molecular phylogenies and host-parasite cospeciation. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **349**, 129–143.
Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W. and Nadler, P.A. (1994). Disparate rates of molecular evolution in cospeciating hosts and their parasites. *Science* **265**, 1087–1090.
Harvey, P.H. and Keymer, A.E. (1991). Comparing life histories using phylogenies. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **332**, 31–39.
Harvey, P.H. and Pagel, M. (1991). *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
Harvey, P.H., Read, A.F. and Nee, S. (1995). Why ecologists need to be phylogenetically challenged. *Journal of Ecology* **85**, 535–536.
Hugot, J.-P. (1999). Primates and their pinworm parasites: the Cameron hypothesis revisited. *Systematic Biology* **49**, 523–546.
Legendre, P. and Legendre, L. (1998). *Numerical Ecology*. Elsevier, Amsterdam.
Leroi, A.M., Rose, M.R. and Lauder, G.V. (1994). What does the comparative method reveal about adaptation? *American Naturalist* **143**, 381–402.
Littlewood, D.J.T. and Bray, R.A. (2001). *Interrelationships of the Platyhelminthes*. London, Taylor and Francis.
Losos, J.B. (1994). An approach for the analysis of comparative data when a phylogeny is unavailable or incomplete. *Systematic Biology* **43**, 117–123.
Martins, E.P. (1996). Conducting phylogenetic comparative studies when phylogeny is not known. *Evolution* **50**, 12–22.
Martins, E.P. and Garland, T. Jr. (1991). Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. *Evolution* **45**, 534–557.
McKitrick, M.C. (1993). Phylogenetic constraint in evolutionary theory: has it any explanatory power? *Annual Review of Ecology and Systematics* **24**, 307–330.
Morand, S. (1996). Life-history traits in parasitic nematodes: a comparative approach for the search of invariants. *Functional Ecology* **10**, 210–218.
Morand, S. (2000). Wormy world: comparative tests of theoretical hypotheses on parasite species richness. In: *Evolutionary Biology of Host-Parasite Relationships: Theory Meets Reality* (R. Poulin, S. Morand and A. Skorping, eds), pp. 63–79. Amsterdam: Elsevier.
Morand, S. and Harvey, P.H. (2000). Mammalian metabolism, longevity and parasite species richness. *Proceedings Royal Society London B* **267**, 1999–2003.
Morand, S. and Poulin, R. (1998). Density, body mass and parasite species richness of terrestrial mammals. *Evolutionary Ecology* **12**, 717–727.
Morand, S., Legendre, P., Gardner, S.L. and Hugot, J.-P. (1996). Body size evolution of oxyurid (Nematoda) parasites: the role of hosts. *Oecologia* **107**, 274–282.
Morand, S., Page, R.D.M., Hafner, M.S. and Reed, D.L. (2000). Comparative evidence of host-parasite coadaptation: body-size relationships in pocket gophers and their chewing lice. *Biological Journal of the Linnean Society* **70**, 239–249.

Murphy, W.J., Eizirik, E., Johnson, W.E., Ping Zhang, Y., Ryder, O.A. and O'Brien, S.J. (2001). Molecular phylogenetics and the origins of placental mammals. *Nature* **409**, 614–616.

Orme, C.D.L., Isaac, N.J.B. and Purvis, A. (2002a). Are most species small? Not within species-level phylogenies. *Proceedings of the Royal Society of London B* **269**, 1279–1287.

Orme, C.D.L., Quicke, D.L.J., Cook, J.M. and Purvis, A. (2002b). Body size does not predict species richness among the metazoan phyla. *Journal of Evolutionary Biology* **15,** 235–247.

Poulin, R. (1995). Phylogeny, ecology, and the richness of parasite communities in vertebrates. *Ecological Monographs* **65**, 283–302.

Poulin, R. (1998). *Evolutionary Ecology of Parasites: from Individuals to Communities*. London, Chapman & Hall.

Poulin, R. (2002). The evolution of monogenean diversity. *International Journal for Parasitology* **32**, 245–254.

Poulin, R. and Morand, S. (2000). The diversity of parasites. *Quarterly Review of Biology* **75**, 277–293.

Price, T. (1997). Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **352**, 519–529.

Purvis, A. and Garland, T. Jr. (1993). Polytomies in comparative analyses of continuous characters. *Systematic Biology* **42**, 569–575.

Purvis, A. and Rambaut, A. (1995). Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Computer Applications for the Biosciences* **11**, 247–251.

Purvis, A., Gittleman, J.L. and Luh, H.-K. (1994). Truth or consequences: effects of phylogenetic accuracy on two comparative methods. *Journal of Theoretical Biology* **167**, 293–300.

Rees, M. (1995). EC-PC comparative analyses. *Journal of Ecology* **83**, 891–893.

Ricklefs, R.R. and Starck, J.M. (1996). Applications of phylogenetically independent contrasts: a mixed progress report. *Oikos* **77**, 167–172.

Simková, A., Desdevises, Y., Gelnar, M. and Morand, S. (2000). Coexistence of gill ectoparasites (*Dactylogyrus*: Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. *International Journal for Parasitology* **30**, 1077–1088.

Skorping, A., Read, A.F. and Keymer, A.E. (1991). Life history covariation in intestinal nematodes of mammals. *Oikos* **60**, 365–372.

Sorci, G., Morand, S. and Hugot, J.-P. (1997). Host-parasite coevolution: comparative evidence for covariation of life-history traits in primates and oxyurid parasites. *Proceedings of the Royal Society of London B* **264**, 285–289.

Symonds, M.R.E. (2002). The effects of topological inaccurrency in evolutionary trees on the phylogenetic comparative method for independent contrasts. *Systematic Biology* **51**, 541–543.

Westoby, M., Leishman, M.R. and Lord, J.M. (1995a). On misinterprinting the 'phylogenetic correction'. *Journal of Ecology* **83**, 531–534.

Westoby, M., Leishman, M.R. and Lord, J.M. (1995b). Further remarks on 'phylogenetic correction'. *Journal of Ecology* **83**: 727–730.

Westoby, M., Leishman, M.R. and Lord, J.M. (1995c). Issues of interpretation after relating comparative datasets to phylogeny. *Journal of Ecology* **83**, 892–893.