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Source: *Philosophical Transactions: Biological Sciences*, Vol. 349, No. 1327, New Uses for New Phylogenies (Jul. 29, 1995), pp. 77-83
Published by: Royal Society
Stable URL: http://www.jstor.org/stable/56126
Accessed: 19-01-2017 14:05 UTC

Molecular phylogenies and host–parasite cospeciation: gophers and lice as a model system

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SUMMARY

Recent methodological advances permit a rigorous comparison of phylogenetic trees for hosts and their parasites to determine the extent to which these groups have cospeciated through evolutionary time. In cases where significant levels of cospeciation are indicated, comparison of amounts of evolutionary change that have accumulated along analogous branches in the host and parasite trees provides a direct assessment of relative rates of evolution in the two groups. For such a comparison to be meaningful, the features compared in the hosts and parasites should be genetically based, evolutionarily homologous, and should evolve in a roughly time-dependent fashion within each group. Nucleotide sequences encoding homologous genes in hosts and parasites are an ideal source of data for comparative studies of evolutionary rates. Recent studies of pocket gophers and their lice are used to illustrate the variety of questions that can be addressed through phylogenetic study of host–parasite systems.

1. INTRODUCTION

This paper outlines a general theoretical and methodological framework for comparing phylogenies of hosts and parasites to address a broad variety of evolutionary questions that could not otherwise be investigated by independent study of either group. The rationale, advantages and limitations of this approach (known generally as the comparative method) have been discussed elsewhere (Harvey & Pagel 1991), as has the long and illustrious history of host–parasite studies in general (Brooks & McLennan 1993). In this paper we illustrate how the application of new and powerful molecular techniques to comparative studies of host–parasite phylogeny enables the study of an entirely new domain of topics that could not be explored with non-molecular data.

Host–parasite systems are intrinsically interesting to evolutionary biologists because they signal a long and intimate association between two or more groups of organisms that are distantly related and quite dissimilar biologically. This long history of association often leads to reciprocal adaptations in the hosts and their parasites (classical coevolution or coadaptation) as well as contemporaneous cladogenic events in the two lineages (cospeciation). The phenomenon of cospeciation is of particular interest to comparative phylogeneticists because cospeciation events identify temporal links between the host and parasite phylogenies, and thus provide an internal time calibration for comparative studies of rates of evolution in the two groups. Evidence of cospeciation also can be used to test hypotheses of coadaptation in the hosts and parasites.

Although evidence of cospeciation in a host–parasite assemblage presents exciting opportunities for the study of evolution, the task of obtaining this evidence is fraught with theoretical and methodological challenges. The analysis involves three steps (tree building, tree comparison, and estimation of divergence), each of which is dependent on the prior step, and each of which requires a different set of experimental and analytical tools. To illustrate this three-tiered protocol for investigation of cospeciation, we use the example of pocket gophers and their chewing lice studied by Hafner *et al.* (1994).

2. POCKET GOPHERS AND THEIR LICE

The hosts in this example include several species of pocket gophers of the rodent family Geomyidae. Pocket gophers are fossorial and extremely asocial, and gopher species generally are allopatric. Nearly all species of pocket gophers are parasitized by chewing lice of the mallophagan family Trichodectidae. These lice are restricted to pocket gophers, and the entire life cycle of these wingless insects occurs on the host. Thus the combined biological characteristics of pocket gophers and chewing lice (i.e. asocial hosts, well-dispersed host populations, and parasites with low vagility) suggest that the lice have few opportunities for colonization of new host species (Nadler & Hafner 1989; Nadler *et al.* 1990). This, in turn, has resulted in a high level of cospeciation in this host–parasite assemblage (Hafner & Nadler 1988).

Cospeciation in pocket gophers and their chewing lice has been investigated from a variety of perspectives, including morphology (Timm 1983), allozymes (see, for example, Hafner & Nadler 1988; Demastes & Hafner 1993), and nucleotide sequences (Hafner *et al.* 1994). In each case, evidence of cospeciation in this assemblage has been so dramatic that the gopher-louse

Phil. Trans. R. Soc. Lond. B (1995) **349**, 77–83
Printed in Great Britain

system has become literally a 'text-book example' of cospeciation (see, for example, Noble *et al.* 1989; Esch & Fernández 1993; Ridley 1993). Most recently, Hafner *et al.* (1994) obtained DNA sequences (379 base pairs) from the same region of the cytochrome *c* oxidase subunit I (COI) gene from the mitochondria of 15 taxa of pocket gophers and 17 taxa of lice that parasitize these gophers. For details about the taxa, data set and analysis, the reader is referred to the paper by Hafner *et al.* Here we focus on this study to illustrate our general method of investigation and to demonstrate the utility of molecular data for the study of cospeciation.

3. RECONSTRUCTING HOST AND PARASITE PHYLOGENIES

The development of phylogenetic trees for the hosts and their parasites lays the foundation for subsequent tests of cospeciation. Because further analyses are dependent on the quality of these trees, the trees must be consistent, well-resolved and independent (i.e. one phylogeny cannot be inferred from the other (Hafner & Nadler (1990)). Furthermore, if one intends to study comparative rates of molecular evolution, the trees must be based on genetic systems that are homologous in the hosts and parasites (such as the COI gene in gophers and lice compared by Hafner *et al.* (1994)).

Systematists have developed a large number of methods for estimating phylogenies (Swofford & Olsen 1990; Hillis *et al.* 1993), each of which uses a different model of character evolution and potentially yields a different tree for the group studied. Because no single method of phylogenetic analysis is universally regarded as superior to others, it behoves the investigator to use multiple methods and to indicate how different analyses affect tree structure. Importantly, demonstration that the host and parasite phylogenies are reasonably robust to different methods of analysis will increase confidence in subsequent tests of cospeciation.

Hafner *et al.* (1994) used four tree-building methods to reconstruct gopher and louse relationships and showed that major portions of the phylogenies were insensitive to method of analysis. Nevertheless, the multiple analyses revealed phylogenetic uncertainty in certain regions of the host and parasite trees, which caused Hafner *et al.* to retain multiple trees (four host trees and five parasite trees) for subsequent tests of cospeciation. Because all systematic analyses will be hampered by some degree of phylogenetic uncertainty, and because the source of that uncertainty generally is unknown (limitations of the data, weakness of the analysis, or both), we recommend the use of multiple host and parasite trees for tests of cospeciation in all but the most clear-cut cases. To simplify the following discussion, we shall restrict our analysis to the gopher and louse phylogenies illustrated in figure 1 (data from Hafner *et al.* 1994).

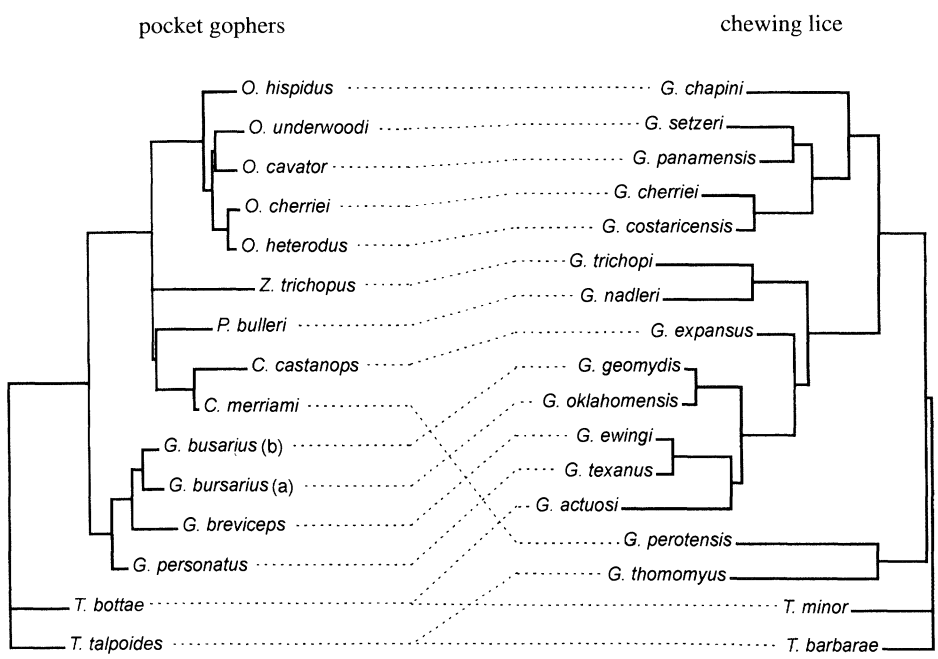

Figure 1. Phylogenies for pocket gophers and their chewing lice based on nucleotide sequence data analysed by Hafner *et al.* (1994). Shown are composite trees based on multiple methods of phylogenetic analysis detailed by Hafner *et al.* (1994). Branch lengths are proportional to expected numbers of substitutions at the third codon position in the COI gene estimated by using Felsenstein's (1989) maximum-likelihood algorithm (DNAML, with transition/transversion ratio of 4.0 for both clades). Coexisting hosts and parasites are connected by dashed lines. Pocket gopher genera are *Orthogeomys*, *Zygogeomys*, *Pappogeomys*, *Cratogeomys*, *Geomys* and *Thomomys*. *Geomys bursarius* is represented by two subspecies ((a) is *G. b. halli*; (b) is *G. b. majusculus*). Chewing louse genera are *Geomydoecus* and *Thomomydoecus*.

4. RECONSTRUCTING THE HISTORY OF A HOST–PARASITE ASSOCIATION

The prerequisite for any comparison of host–parasite evolution is a reconstruction of the history of that association. Here, the challenge is to determine whether the degree of similarity observed between the host and parasite phylogenies exceeds the similarity we would expect to see by chance. At present there are two methods for obtaining such a reconstruction, Brooks's parsimony analysis (Brooks & McLennan 1991) and Page's (1990*a*, 1993*a*, 1994) component analysis. Brooks's parsimony analysis uses additive binary coding to represent parasite phylogenies, then optimizes the resulting codes on the host phylogeny. Page (1990*a*, 1994) has argued that this procedure can give spurious results; hence in this study we use the most recent refinement of component analysis (Page 1995).

Component analysis is a method developed originally to reconstruct biogeographical histories of taxa (Nelson & Platnick 1981), but its similarity to the procedure of Goodman *et al.* (1979) for comparing gene trees and species trees suggests that component analysis is sufficiently general to be applied to any historical association, including host–parasite systems (Page 1990*a*). So far, component analysis has been applied to the association of the tree genus *Nothofagus* and its fungal parasite *Cyttaria* (Page 1990*a*), pocket gophers and their lice (see, for example, Page 1990*b*; Hafner *et al.* 1994), and seabirds and their lice (Paterson *et al.* 1993; Paterson 1994).

The analogy between comparing parasite and host phylogenies, and comparing gene and organismal phylogenies is instructive. Parasitologists have generally assumed that unless host and parasite phylogenies are absolutely congruent, host-switching has occurred (see, for example, Brooks & McLennan 1991, p. 205). The complexity of the relationship between gene trees and species trees, even in the absence of horizontal transfer (such as introgression), suggests that this assumption may be unjustified. For example, if we view parasites as 'genes' of their hosts, passed from parent to offspring for multiple generations, we can imagine that the parasites might be subject to the same stochastic processes that affect genes in populations, such as loss through drift, retention of ancestral (plesiomorphic) character states, and lineage sorting (Avise *et al.* 1984). If so, it is likely that much of the incongruence between host and parasite trees may result simply from chance loss or retention of parasite lineages. Further, we might expect to see higher levels of incongruence between host and parasite phylogenies when younger lineages are studied (e.g. closely related species) simply because there has been insufficient time for lineage sorting of the parasites. In theory, chance extinction of the parasites should result eventually in reciprocal monophyly of parasite lineages on sister taxa of hosts (Demastes & Hafner 1993).

(*a*) *Has cospeciation occurred?*

A simple test of the hypothesis of cospeciation is to ask whether the structure of the parasite tree is independent of that of its host. If so, we would expect the amount of cospeciation observed between the hosts and parasites (i.e. the number of cospeciation events in the two phylogenies) to be no greater than that expected between the host tree and random parasite trees (Page 1995). Applying this test to the phylogenies in figure 1, we reject the hypothesis that the louse phylogeny is independent of the gopher phylogeny ($p = 0.004$, computed by using 1000 random trees). It is possible that recent host switching could produce spurious congruence between the host and parasite trees, especially if the parasites preferentially colonized hosts that are closely related. Similarly, incongruence between the host and parasite phylogenies could result from differential survival of multiple parasite lineages, rather than host switching (as discussed above; see also Page (1993*b*)). If genetic data are available for hosts and parasites, as in our gopher-louse example, information on amounts of genetic divergence (or relative coalescence times) can assist our efforts to discriminate between these possibilities (Page 1993*b*).

5. STUDIES OF COADAPTATION AND COLONIZATION IN HOSTS AND PARASITES

Component analysis (Page 1993*a*, 1995) identifies pairs of equivalent nodes in the host and parasite trees that reflect the same historical event. These equivalent nodes can be depicted visually by overlaying the parasite tree on the host tree, wherein each node of the parasite tree is adjacent to the corresponding node in the host tree (figure 2). Hypotheses of coadaptation in the hosts and parasites can be tested using these nodes. For example, Harvey & Keymer (1991) used simplified phylogenies of gophers and lice taken from Hafner & Nadler (1988) to show that evolution of body size in lice and their hosts is highly correlated. Numerous other morphological, physiological, and ecological attributes of the hosts and parasites can be compared by using the cospeciation framework.

In a parasite clade that shows evidence for host switching, the investigator may wish to ask if there are geographical, morphological or ecological correlates of host switching. Reconstruction of the biogeographical history of host-switching events may reveal whether colonization of new hosts is simply opportunistic (nearest neighbour), or whether parasites are tracking a particular resource in the host taxa that is not itself correlated with host phylogeny (such as quill size preferences shown by the feather mites of birds (Kethley & Johnston 1975)). Knowledge of past host-switching events, coupled with genetic data for the hosts and parasites, permits the detection of possible changes in rates of evolution in the hosts or parasites (or both) after colonization events.

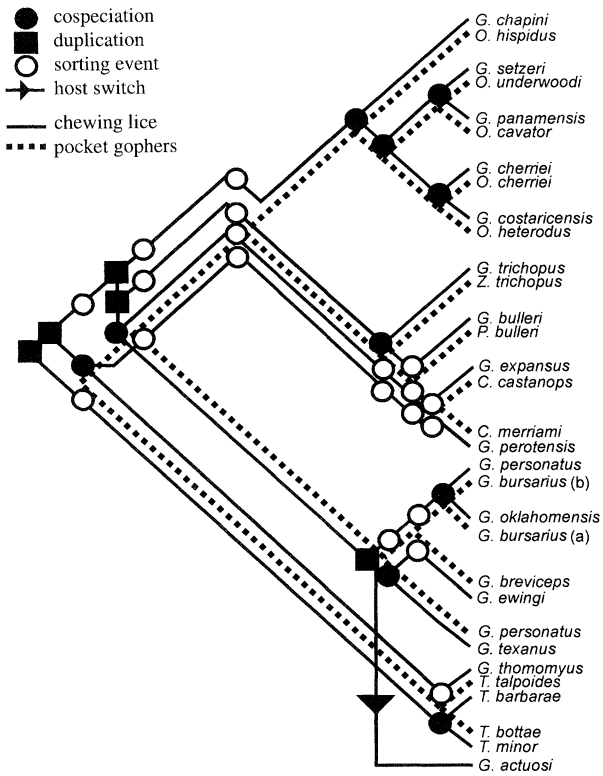

Figure 2. A possible reconstruction of the history of the gopher-louse association that postulates 10 cospeciation events, 5 duplications (*in situ* speciation of the lice on the same host), 20 sorting events (instances where louse lineages have been lost or remain undetected), and a single host switch (by *Geomydoecus actuosi*).

6. COMPARISONS OF GENETIC DIVERGENCE IN HOSTS AND PARASITES

There are many ways to convert molecular data (including data from allozymes, restriction-fragment patterns, and protein and DNA sequences) into estimates of genetic divergence (Swofford & Olsen 1990). Each method has inherent advantages and limitations, and each involves assumptions about the nature of evolutionary change at the molecular level. Recent comparative studies of genetic differentiation in hosts and parasites have used either pairwise estimates of genetic distance (see, for example, Hafner & Nadler 1990; Page 1990*a*) or estimates of length of homologous branches in the host and parasite trees (see, for example, Hafner *et al.* 1994). The former method, although easy to apply, has fundamental statistical limitations because of the non-independence of pairwise measurements. The latter method (branch length comparisons) generally avoids the problem of statistical dependence, but requires an often complex model of evolution to apportion change onto branches. As we shall illustrate later, different models often yield different estimates of branch lengths, which may result in different interpretations of relative rates of change in the hosts and their parasites. Until knowledge of molecular evolution advances to the point where generally accepted models are available, the researcher should be explicit about the model selected and should be aware of the implications of that model.

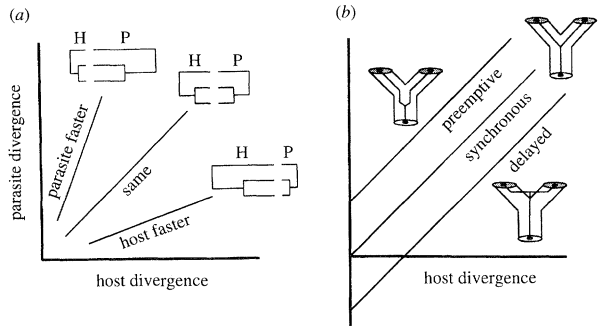

Figure 3. Bivariate plots of the relationship between parasite divergence and host divergence. The slope of the relationship (*a*) indicates relative rates of evolution in the two clades. The trees (inset in (*a*)) are drawn with branch lengths proportional to amount of genetic change in the hosts (H) and parasites (P). The *y*-intercept (*b*) indicates the relative timing of speciation events. The inset figures in (*b*) illustrate relative timing of speciation events in the hosts (outer portion of figure) and their parasites (thin line within each figure). Modified from Hafner & Nadler (1990, fig. 2).

(*a*) *Molecular clocks and relative timing of cospeciation events*

Once estimates of branch lengths have been calculated, lengths of equivalent branches in the host and parasite trees can be compared. Although the comparison may seem straightforward, the interpretation of differences in branch length may be confounded by multiple factors. For example, host branches may be consistently longer than parasite branches because the hosts are evolving more rapidly, or because the hosts consistently diverged before their parasites, or both. Thus meaningful interpretation of this comparison requires knowledge of relative rates of change in the hosts and parasites, which assumes that genetic change in each group is roughly clocklike. Our reliance on molecular clocks for this part of the analysis requires that we test for the existence of a clock, rather than simply assume that one exists. Various tests are available for this purpose (see, for example, Muse & Weir 1992; Goldman 1993; Adell & Dopazo 1994).

Hafner & Nadler (1990) proposed a framework for comparing host and parasite divergence, given molecular clocks (which may tick at different rates) in both groups. Fitting a line to a plot of parasite divergence against host divergence (figure 3) allows us to describe simultaneously two aspects of host–parasite divergence. The slope of the line (figure 3*a*) is an estimate of the relative rate of genetic change in the two groups. The *y*-intercept of the line (figure 3*b*) measures genetic divergence in the parasites at the time of host speciation. For example, an intercept of zero indicates synchronous cospeciation, wherein hosts and parasites speciate simultaneously. A negative intercept suggests delayed cospeciation, in which case the parasites tend to speciate consistently after their hosts. Finally, a positive intercept signals preemptive cospeciation, in which case the parasites diverge before their hosts.

Returning to the analogy with gene trees, if the bivariate plots shown in figure 3 were instead plotting sequence divergence for a given gene against time of taxon divergence, a positive intercept would reflect the

average sequence divergence that exists among populations of a species (Lynch & Jarrell 1993). By analogy, a positive intercept in the comparison of host–parasite sequence divergence would reflect average differentiation among parasite populations of a single host species. Thus we might expect the intercept to be positive in situations where parasite populations living on different hosts of the same species are genetically divergent. It is perhaps significant, therefore, that louse populations living on different individual hosts at a single locality show moderately high levels of genetic divergence (Nadler *et al.* 1990). Whether or not this population-level differentiation has long-term evolutionary consequences has yet to be explored.

(*b*) *Estimating branch lengths*

There are many advantages to using DNA sequence data in studies of host–parasite cospeciation. Among these is the fact that the characters being compared have a known genetic basis. In contrast, morphological characters may be polygenic or lack a genetic basis altogether. With DNA sequence data we also are able to compare homologous sequences in the hosts and parasites, thereby avoiding the problem of comparing non-homologous morphological characters, or allozyme characters of dubious homology. Finally, DNA sequence data are relatively easy to generate and potentially provide a large number of characters for high-resolution analyses.

If we consider the gopher-louse data, most substitutions in the gopher and louse COI sequences are synonymous (silent) substitutions at the third codon position (Hafner *et al.* 1994). In fact, third-position substitutions are so numerous that almost any pairwise comparison will suffer from the effects of multiple substitutions at the same nucleotide position. Unless corrected for, this substitutional saturation will lead to an underestimate of the genetic distance between taxa (or underestimates of branch lengths), which is why multiple methods have been developed to compensate for the effects of saturation (Tajima & Nei 1984). We should also note that if the substitution process differs at different sites along the sequence (e.g. first, second and third codon positions), then the utility of a single overall measure of sequence divergence is dubious (Irwin *et al.* 1991).

Although it is widely acknowledged that estimates of DNA sequence divergence should be adjusted for the effects of saturation, there is no general consensus as to how this should be done. For example, Hafner *et al.* (1994) attempted to correct for transition bias in the gopher and louse COI data by using the largest observed pairwise transition bias in a maximum-likelihood phylogeny reconstruction. They reasoned that this value, which is usually measured between the most recently diverged taxa, is least likely to be affected by saturation and is therefore the most reasonable estimate of the actual transition bias for this gene region. In contrast, Page (in preparation) recommends the use of the transition bias estimate that maximizes the likelihood of the phylogeny. The use of these different correction factors can have a profound influence on estimates of branch length. For example, the analysis by Hafner *et al.* suggests that lice are evolving 10–11 times more rapidly than their hosts at selectively neutral sites. In contrast, Page's analysis suggests that lice are evolving only twice as fast as gophers. Research into the effects of transitional saturation (and evolutionary models, in general) is now moving at a rapid pace (Goldman 1993; Yang 1994), and we expect that some degree of consensus will be reached in the near future.

(*c*) *Phylogenetic sampling*

Another correlate of the accuracy of branch length estimation is phylogenetic sampling. The denser the sampling of lineages, the greater the chances of detecting evolutionary change (Langley & Fitch 1974; Moore *et al.* 1976; Fitch & Bruschi 1987; Fitch & Beintema 1990). In the gopher-louse study (Hafner *et al.* 1994), the 17 louse species examined tend to represent single exemplars from larger clades containing a total of 122 recognized species (Page *et al.* 1995). Pocket gophers are also taxonomically diverse (approximately 40 species and 450 subspecies), and relatively few taxa have been examined from a molecular perspective. Ideally, future studies will involve exhaustive sampling of gopher and louse clades so that different lineages within each group can be compared to determine whether there are lineage-specific molecular clocks. The DNA data from Hafner *et al.* (1994) suggest that there may be lineage-specific rate differences, although these deviations may result from sampling error (Page, in preparation).

(*d*) *Stochasticity*

The DNA sequences analysed by Hafner *et al.* (1994) represent relatively short regions (379 b.p.) of a single mitochondrial gene (COI). As a result, extrapolation from these data to the entire COI gene, or to the entire mitochondrial genome, are tenuous. In addition, random events, such as lineage sorting (Avise *et al.* 1984) may have resulted in a mitochondrial genealogy ('gene tree') that is quite different from the nuclear genealogy ('species tree'). Thus it is important for researchers studying organellar genomes to compare their phylogenies with those based on nuclear-encoded characters (e.g. nuclear sequences, morphology or allozymes). So far, the nuclear and mitochondrial phylogenies for gophers and lice are in close agreement (Hafner & Nadler 1988). However, we expect to see increased discordance between mitochondrial and nuclear genealogies as we explore cospeciation on a finer scale (e.g. within species). For example, Patton & Smith (1994) have shown that the mitochondrial and allozyme trees for pocket gophers of the genus *Thomomys* are incongruent. If chewing lice are transmitted primarily from mother to offspring in *Thomomys* (as are mitochondrial haplotypes), then we predict that the phylogeny of lice from *Thomomys* will be more similar to the host mitochondrial tree than the nuclear tree (Nadler *et al.* 1990). We are currently testing this hypothesis.

Because of the relatively small number of nucleotides sampled in the gopher-louse study, maximum-likelihood confidence limits on the estimates of branch lengths are quite broad, hampering efforts to compare host and parasite evolution. Although sampling error (both genome sampling and taxon sampling) certainly contributes to this decreased resolution, it is also likely that stochasticity of the substitution process and clade-specific variation in rates of substitution decrease our ability to see clear, assemblage-wide trends. Where general trends are evident (e.g. the gopher-louse rate difference reported by Hafner *et al.* (1994)), they are not particularly strong. Clearly, more sequence data and increased taxonomic sampling are needed to increase our confidence in these preliminary findings.

7. CONCLUSIONS AND PERSPECTIVES

Gillespie (1991, p. 139) distinguishes between two uses of molecular clocks: as a source of data on times of divergence between lineages (coalescence times), or as tests of hypotheses about molecular evolution. Similarly, we can use measures of molecular divergence to test our reconstructions of host and parasite phylogenies (and to calculate time since divergence of cospeciating clades), or we can endeavour to probe in detail the mechanics of molecular evolution and evolutionary rates in the hosts and parasites. The latter approach has more general appeal because it has the potential to yield findings that transcend the particular host–parasite system studied. For example, discovery of rate correlates or other evolutionary patterns shared between distantly related hosts and parasites (e.g. mammals and insects in the study by Hafner *et al.*) may signal underlying evolutionary processes that have a high degree of universality. In this regard, T. Spradling (in M.S.H.'s laboratory) is currently sequencing the COI gene of whipworms (endoparasitic nematodes) that parasitize pocket gophers. If cospeciation is evident in all three symbionts (gophers, lice and whipworms), this framework can be used to test for rate correlates or other evolutionary patterns that show even greater universality.

Future studies comparing population structure of hosts and their parasites will reveal whether the structuring of a parasite population on an individual host (and founder events as new hosts are colonized) tend to accelerate long-term parasite evolution relative to that of their hosts (Nadler *et al.* 1990). To be convincing, such a test will have to demonstrate that short-term population-level phenomena (such as decreased heterozygosity and polymorphism in the parasites) have persistent and long-term phylogenetic consequences. Similarly, studies of the molecular genetics of parasites at zones of hybridization between host taxa can yield important information about the history of the zone (see, for example, Patton *et al.* 1984; Nadler *et al.* 1990) or about modes of parasite transmission (J. Demastes, in preparation). If genetic introgression is present in both groups, then the rate and pattern of introgression can be compared to reveal common demographic patterns. In other cases, parasites can be treated as 'genes' of their hosts to serve as an independent measure of extent of host introgression (Bohlin & Zimmerman 1982, Patton *et al.* 1984).

Although, at present, there are few published studies of cospeciation explored from a molecular perspective, we expect rapid growth in this research area as molecular tools become more widely available and the advantages of this approach better known. Unfortunately, many host–parasite systems will show little or no evidence of cospeciation (see, for example, Baverstock *et al.* 1985), which will preclude comparative studies of higher-order phenomena such as evolutionary rates. However, in systems with appreciable cospeciation, the researcher will have the unparalleled opportunity to compare evolution in the same gene(s), over the same period of time, in distantly related organisms. Within this framework, the potential is great for the discovery of large-scale evolutionary patterns that apply to diverse groups of organisms.

We thank Paul Harvey for inviting us to the Royal Society conference. Dale Clayton, James Demastes, Theresa Spradling and Xuhua Xia provided helpful comments on the manuscript. This research was supported in part by a National Science Foundation grant to MSH.

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