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COSPECIATION IN HOST-PARASITE ASSEMBLAGES: COMPARATIVE ANALYSIS OF RATES OF EVOLUTION AND TIMING OF COSPECIATION EVENTS

MARK S. HAFNER AND STEVEN A. NADLER[1]

Museum of Natural Science and Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

Abstract.—Documentation of widespread cospeciation in a host-parasite assemblage requires statistical evidence that the congruence observed between the host and parasite phylogenies exceeds that expected by chance. Although the validity of this test rests on the assumption of independence of the host and parasite phylogenies, this critical assumption may be violated in many tests of cospeciation. Herein, we emphasize the need for rigorous tests of cospeciation in host-parasite assemblages, and we show how estimates of genetic distance can be used to investigate relative rates of evolution and timing of phylogenesis in the hosts and parasites once widespread cospeciation has been documented for the assemblage. The method involves a nonparametric test of association between genetic-distance matrices for the hosts and their parasites. If the association is statistically significant, the relationship is examined in greater detail using bivariate analysis. We use an example from our studies of pocket gophers and chewing lice to illustrate how genetic distances can be used to explore relative rates of genetic change in the two groups and to investigate relative timing of cospeciation events in the assemblage. [Cospeciation; host-parasite coevolution; rates of evolution; genetic distances; pocket gophers; chewing lice.]

Within the broad context of host-parasite coevolutionary theory, the inference of cospeciation is considered appropriate for a given host-parasite assemblage if the hosts and their parasites show identical patterns of phylogenetic differentiation. In practice, however, identical patterns of phylogenesis in hosts and their parasites are only rarely observed. Thus, in most studies of cospeciation, the investigator must accept a certain degree of discordance between host and parasite phylogenies and ask whether evidence of cospeciation is *widespread* within the assemblage. The search for widespread cospeciation usually involves a general assessment of congruence between host and parasite phylogenies or between host and parasite taxonomic boundaries or geographic distributions (e.g., Kethley and Johnston, 1975; Kim et al., 1975; Eveleigh and Amano, 1977; Brooks, 1979; Fain, 1979; Moss, 1979; Radovsky, 1979; Hellenthal and Price, 1984). In comparisons of host and parasite genealogical trees, the inference of widespread cospeciation usually involves subjective appraisal of branching similarity, wherein a high level of congruence is assumed to reflect widespread cospeciation of hosts and their parasites, and a low level of congruence indicates that the majority of observed host-parasite associations cannot be attributed to parallel phylogenesis. Until recently (Humphries et al., 1986; Brooks, 1987; Simberloff, 1987; Page, 1989, 1990), few workers have quantified similarity between host and parasite phylogenies. Because the inference of widespread cospeciation is unwarranted if the observed similarity can be explained by chance, it is critical that investigations of cospeciation include assessments of the statistical significance of topological similarity of host and parasite phylogenetic trees.

Even more fundamental to documentation of cospeciation is the logical and statistical requirement for independence of

[1] Present address: Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115.

the host and parasite phylogenies. For this reason, uncritical comparison of published taxonomies of hosts and their parasites to investigate cospeciation may be imprudent; the taxonomy of one group may have been influenced, explicitly or implicitly, by knowledge of relationships within the other. Because systematic investigations of parasites generally postdate systematic studies of their hosts, parasite taxonomies are especially subject to influence from prior investigations of the hosts. In addition, parasitological dogma maintains the expectation of widespread cospeciation in host-parasite assemblages (embodied in Farenholz' Rule; Eichler, 1948), which may bias taxonomic decisions to create an artificial pattern of cospeciation. Thus, in any study of cospeciation, it is critical to document that the parasite phylogeny was constructed without reference to host relationships; only then can the host and parasite phylogenies be compared statistically to investigate the hypothesis of widespread cospeciation.

In theory, two kinds of evidence are necessary (and sufficient) to document widespread cospeciation in a host-parasite assemblage: evidence that the host and parasite phylogenies are derived independently and statistical evidence that the topological similarity of the host and parasite trees exceeds chance expectations. If the host-parasite assemblage in question meets both of these requirements, then the probability of spurious congruence between the trees (which, in theory, could result if the hosts and parasites differentiate at different times but happen to show similar patterns of phylogenesis) is remote. Although documented cases of widespread cospeciation are rare, they are of unusual interest because they reveal a historical linkage between two groups of organisms that invariably have fundamentally different life histories. The temporal aspect of this linkage (i.e., the inference that a pair of hosts diverged at approximately the same time as their parasites) permits examination of relative rates of evolution in the two groups by comparing the amount of evolutionary change (however measured) that each has undergone during the period of parallel phylogenesis. Thus, studies of cospeciating host-parasite assemblages permit examination of a broad spectrum of questions relating to the possible affects of life style, breeding structure, generation time, and other life-history parameters on rates of evolutionary change.

A GENERAL METHOD FOR THE STUDY OF COSPECIATION

Estimates of Genetic Differentiation and Construction of Genealogical Trees

The procedure we outline for the study of cospeciation requires independent assessments of genetic differentiation in a group of hosts and their parasites. Molecular methods (including protein electrophoresis) are particularly appropriate for such assessments because these methods allow measurement of differentiation in the hosts and their parasites using a similar genetic yardstick. Molecular data can be used to derive phylogenies that are free of assumptions concerning rates of evolutionary change. In addition, molecular data can be converted into estimates of genetic distance that can be used to explore relative amounts of genetic change in the hosts and their parasites and to determine if genetic distances scale roughly in proportion to time (Zuckerkandl and Pauling, 1965; Kimura, 1983).

Given independent molecular data sets for the hosts and their parasites, genealogical trees are constructed from these data for each group. Although the method(s) used to generate the trees will depend on one's systematic philosophy, the investigator should use tree-building algorithms with clearly stated assumptions that can be evaluated by the reader. Because the topology of these trees will be compared statistically to test the hypothesis of widespread cospeciation, the confidence one has in their branching structure will necessarily determine the strength of the inference concerning cospeciation.

Statistical Comparison of Tree Topologies

Next, the genealogical trees for hosts and their parasites are compared to determine

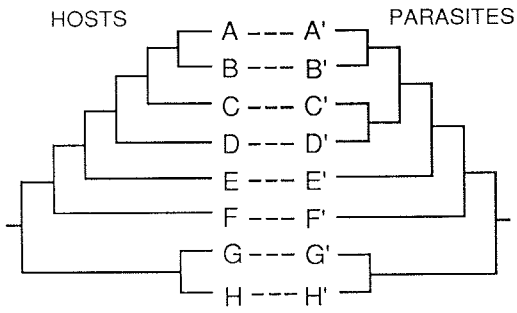

FIG. 1. Hypothetical host and parasite trees, with associated host and parasite taxa linked by dashed lines. Although these trees are not topologically identical, the probability of this degree of similarity occurring by chance is remote ($P < 0.01$).

if their topological similarity exceeds the level of similarity expected by chance. For example, Figure 1 illustrates a pair of hypothetical host and parasite trees that lack complete topological congruence; the trees differ by a single node involving host taxa C and D and their parasites. If the level of topological similarity of these trees exceeds chance expectations, then it is appropriate to infer widespread cospeciation between these hypothetical hosts and parasites. Thus, an assemblage may show a *general* pattern of cospeciation despite clear evidence against cospeciation for certain of the included taxa (e.g., taxa C and D and their parasites in Fig. 1). If similarity of the trees does not exceed chance expectations, then the hypothesis of widespread cospeciation is falsified for this assemblage despite the possibility that certain hosts and their parasites in the assemblage may, in fact, have a history of cospeciation.

Several methods are available to compare tree topologies quantitatively (e.g., Platnick and Nelson, 1978; Rosen, 1978; Nelson and Platnick, 1981; Hendy et al., 1984; Simberloff, 1987; Page, 1989). Although the statistical validity of certain of these procedures has been questioned (Simberloff et al., 1981; Simberloff, 1987), use of quantitative procedures clearly is an improvement over traditional, visual-intuitive methods for comparing trees. Regardless of the method used to evaluate their topological similarity, the hypothetical trees illustrated in Figure 1 are similar beyond chance expectations. For example, using Nelson and Platnick's (1981) method, we determined that the probability of chance similarity between the two trees (Fig. 1) is $P = 0.0012$. Similarly, the method of Hendy et al. (1984) yields a probability of chance similarity of $P = 9.62 \times 10^{-5}$. Thus, these statistical tests falsify the hypothesis of tree independence, and we conclude that cospeciation has been widespread among these hypothetical hosts and their parasites.

Analyses of Evolutionary Rate and Timing

Before proceeding, it is important to reemphasize that the statistical test of tree topologies is both necessary *and* sufficient to document widespread cospeciation in a host-parasite assemblage; the possibility of chance congruence between host and parasite phylogenetic trees has been eliminated on probabilistic grounds. Although documentation of widespread cospeciation is of intrinsic interest in its own right, much more can be learned about the coevolutionary history of the assemblage by comparing degree and pattern of genetic differentiation in the hosts and their parasites. For example, if homologous gene loci or nucleic acid sequences are surveyed in both groups, one can compare interlocus differentiation or sequence-specific rates of evolutionary change in the hosts and parasites. In addition, if the sample of loci or nucleotides surveyed permits robust estimates of genetic distance, one can determine whether the parasites differentiate genetically, on average, more rapidly or more slowly than their hosts.

Although it may seem counterintuitive, documentation of widespread cospeciation in a host-parasite assemblage does not document synchronous speciation events in the two groups; cospeciation requires only that parasite speciation events occur *sometime between* consecutive host speciation events. Parasitological rules (e.g., Manter's Rules; Manter, 1955) predict that most cospeciating host-parasite assemblages will show what we term "delayed cospeciation," wherein parasite speciation lags be-

hind host speciation. Delayed cospeciation might be expected in assemblages in which parasites are more vagile than their hosts, so that extrinsic barriers that block gene flow among host populations will have less immediate impact on gene flow among their parasites. Similarly, delayed cospeciation might be predicted in cases where parasite dispersal is aided by mobile intermediate hosts or vectors, or where passive movement of parasite life stages occurs between allopatric host populations.

The term "synchronous cospeciation" describes those cases in which host speciation and parasite speciation are approximately contemporaneous. Synchronous cospeciation would be expected to occur in assemblages in which the parasites are as vagile as, or less vagile than, their hosts, so that a vicariant event that subdivides a population of hosts causes contemporaneous cessation of gene flow in both hosts and parasites. If this isolation eventually results in speciation in both groups, a pattern of synchronous cospeciation emerges. The possibility of delayed speciation by the host (relative to the parasite), although doubtlessly a real phenomenon in nature, is not considered here because it would usually result in two hosts harboring the same set of parasites. This pattern would not be interpreted as cospeciation, except in the unlikely event that subsequent extinction left only one parasite species on one host and the other parasite species on the other host.

The degree to which a given host-parasite assemblage shows synchronous or delayed cospeciation will depend on biological properties of the hosts and parasites (e.g., their relative vagility, life cycle, population structure, and density) as modified by abiotic factors, including the frequency, magnitude, and duration of vicariant events. The involvement of stochastic factors suggests that different lineages within a single host-parasite assemblage may show different kinds of cospeciation. Nevertheless, if a host-parasite assemblage shows a statistically documented pattern of widespread cospeciation, it would be of interest to know if that pattern consists primarily of delayed or synchronous cospeciation events. This issue can be explored by comparative analysis of genetic distances in the hosts and parasites.

Correlation Analysis Using Genetic Distances

The following analysis is based on the assumption that the host and parasite trees used to test the hypothesis of widespread cospeciation were generated with a tree-building algorithm (such as parsimony analysis) that does not assume homogeneity of evolutionary rates. In this way, the conclusion that a given host-parasite assemblage shows widespread cospeciation (based on the statistical comparison of tree topologies) is not dependent on the assumption of equal rates of genetic change within or between the hosts and parasites. It is legitimate, therefore, to use the same genetic data sets (i.e., those originally used to construct the phylogenetic trees) to investigate whether the hosts and their parasites have undergone equivalent amounts of genetic differentiation during their history of parallel phylogenesis. This question can be addressed by comparing host and parasite genetic distance matrices using a nonparametric test of association, such as the Mantel test (Mantel, 1967; Schnell et al., 1985). This test requires matrices of equal size, which will require elimination of certain host or parasite taxa in the case of redundant parasite distributions (Nelson and Platnick, 1981). In these cases, we recommend iterative tests (with replacement) so that the genetic distances between all coexisting host-parasite pairs can be examined for statistical association. The Mantel test does not require independence among the elements of a matrix; however, recognizing that there is a certain degree of dependence among the elements within a genetic distance matrix, we recommend use of the conservative value of $n - 1$ degrees of freedom (rather than infinite degrees of freedom) in the Mantel test, where n is the number of host (or parasite) taxa per matrix.

A statistically significant relationship between the genetic distance matrices for

hosts and their parasites (using the Mantel test) would be strong evidence for evolutionary rate constancy ("molecular clocks") *within* each group. The alternative (and, in our view, less likely) explanation for the correlation between the distance matrices is that rate fluctuations in one group were mimicked precisely by rate fluctuations in the other. Although a significant relationship between host and parasite genetic distance matrices is consistent with the hypothesis of widespread cospeciation (Hafner and Nadler, 1988), lack of a significant association between the matrices does not falsify the cospeciation hypothesis. The Mantel test can only falsify the hypothesis that there is a significant correlation between amounts of genetic differentiation in the hosts and their parasites; thus, if rates of evolution vary within either or both groups, the test may reveal a nonsignificant relationship. However, regardless of the outcome of the Mantel test, the statistical documentation of widespread cospeciation (based on the initial comparison of tree topologies) remains valid.

A statistically significant elementwise association between the matrices signals a predictable relationship between genetic distances in the two groups, but it does not necessarily indicate *equal* distances. The nature of the relationship between the matrices can be explored by constructing bivariate plots of the corresponding elements in the two matrices (i.e., each pairwise genetic distance for the hosts is plotted against the corresponding genetic distance for their parasites). Although it is statistically invalid to fit a line to these points (because of the dependence among the elements within each matrix), the resulting plot provides a heuristic assessment of the relationship between the two distance matrices. For example, if genetic distances are approximately equal in the two groups, most of the points will lie roughly equidistant from the two axes (Fig. 2a). This pattern would suggest that the average rate of genetic differentiation in the hosts and parasites has been roughly equal during their history of parallel phylogenesis. In contrast, if most of the points lie closer to the host (or parasite) axis, this would indicate that the hosts (or parasites) have differentiated more rapidly than their symbiotic partners. Thus, the bivariate plot enables a general assessment of *between*-group rate differences without resort to an outside time calibration based on fossil or geological evidence.

Regardless of the relative rates of genetic change in the hosts and parasites (i.e., regardless of the slopes of the lines in Fig. 2a), a consistent pattern of synchronous cospeciation will always yield an array of plotted points that emanates from the origin of the graph (Fig. 2b). Similarly, a consistent pattern of delayed cospeciation will depress the array of points uniformly downward (lower their y-intercept) in proportion to the length of the time interval between host speciation and parasite speciation. Thus, different rates of genetic change in the hosts and their parasites should affect only the trajectory of the array, whereas consistent differences in timing of speciation events in the two groups will affect only the y-intercept of the array. Because the factors of rate and timing are effectively decoupled, this method should reveal consistent patterns in timing of cospeciation events regardless of the relative rates of genetic change in the hosts and parasites. Absence of a consistent pattern within an assemblage (i.e., a mixture of synchronous and delayed cospeciation events) will obscure the real relationship between timing of cospeciation events in the hosts and parasites and will usually result in an uninterpretable array of widely scattered points.

AN EXAMPLE: POCKET GOPHERS AND THEIR CHEWING LICE

The hosts in this example include several species of pocket gophers of the rodent family Geomyidae; these fossorial herbivores are asocial, and geomyid species are generally allopatric (Hall, 1981). The pocket gophers are parasitized by chewing lice of the mallophagan family Trichodectidae.

These species of chewing lice are restricted to pocket gophers, and the entire life cycle of these wingless insects occurs on the host. Transmission of chewing lice among pocket gophers appears to require prolonged host-to-host contact (Timm, 1983), which suggests that a major means of louse transfer may be from mother to offspring (mating encounters are brief). Thus, the combined biological characteristics of pocket gophers and chewing lice indicate that the lice have few opportunities for colonization of new host species (Nadler and Hafner, 1989; Nadler et al., 1990).

In previous work (Hafner and Nadler, 1988), we used standard starch-gel electrophoretic procedures to survey allelic variation at 31 protein loci in the pocket gophers (Appendices 1 and 2) and 14 loci in their chewing lice (Appendix 3). Because estimates of genetic distance may be influenced by the ratio of slow : fast-evolving loci surveyed (Sarich, 1977), we surveyed equivalent ratios of relatively slow and relatively fast-evolving loci in the hosts and parasites; 4 of 31 loci (13%) examined in pocket gophers and 2 of 14 loci (14%) surveyed in chewing lice are generally classified as fast-evolving (Kojima et al., 1970; Hafner and Nadler, 1988). To assure that the louse survey was not simply an indirect survey of host tissues contained in the louse's gut, we compared patterns of allelic variation at the 10 loci in common to the two electrophoretic surveys; at all 10 loci, the hosts and parasites had different alleles and different patterns of allelic variation.

Phylogenetic trees for the pocket gophers and their chewing lice are compared in Figure 3. The pocket gopher tree was rooted using the rodent *Dipodomys ordii* (Heteromyidae) as the outgroup, and two chewing lice from mammalian carnivores (*Neotrichodectes mephitidis* and *Trichodectes octomaculatus*) were used to root the chewing louse tree. The trees illustrated were included among the set of shortest trees in parsimony analyses of the protein data (PAUP 3.0; Swofford, 1989). Branch-and-bound searches were used in the parsimony analyses, in which loci were treated as characters and alleles as character states.

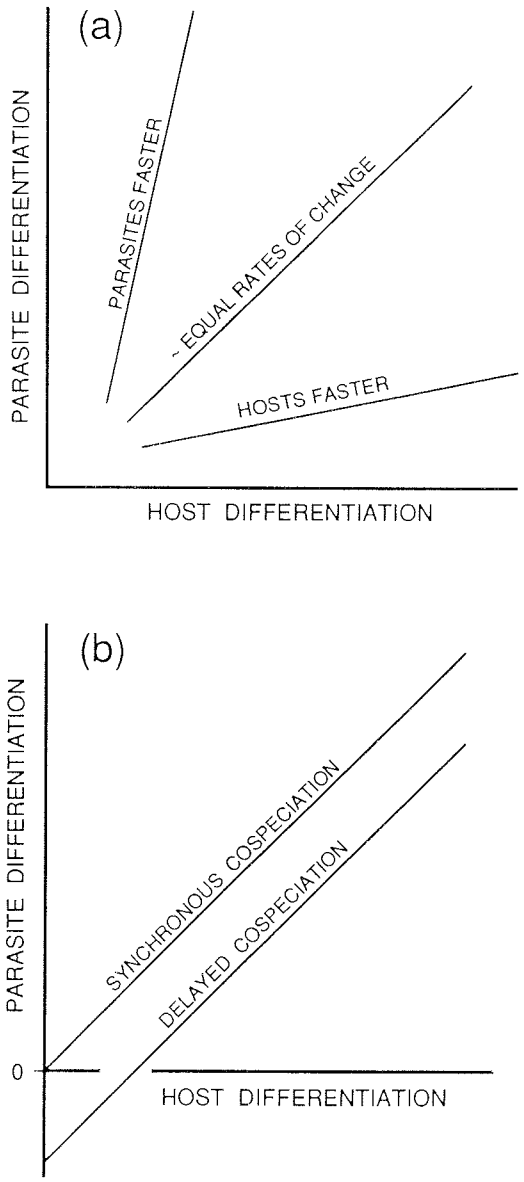

FIG. 2. Hypothetical bivariate plots of corresponding elements in the host and parasite genetic distance matrices. Such plots provide a heuristic assessment of the relationship between corresponding elements of the matrices; the relationship cannot be evaluated statistically because the elements within each matrix are not independent. (a) The trajectory of the plotted points will reflect the general relationship between rates of genetic evolution in the hosts and their parasites. (b) A consistent pattern of delayed parasite speciation (relative to their hosts) is expected to depress the array of points uniformly downward in the graph.

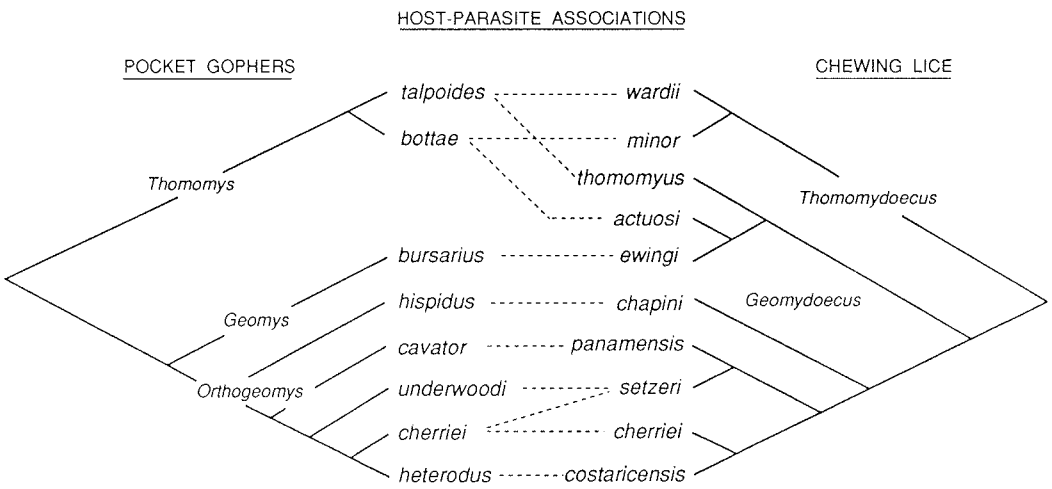

FIG. 3. Pocket gopher and chewing louse phylogenies, with dashed lines indicating host-parasite associations. The branching patterns shown are supported by parsimony (Swofford, 1989), locus-by-locus (Baverstock et al., 1979; Hafner et al., 1987), and UPGMA (Sneath and Sokal, 1973) analyses of the electrophoretic data. Outgroups (not shown) were used to root the trees. Both species of *Thomomys* host two species of lice. The louse *G. setzeri* is known from all populations of the pocket gopher *O. underwoodi* and also occurs in a few populations of the host *O. cherriei*.

The reported tree lengths include changes within terminal taxa.

Parsimony analysis of the pocket gopher data generated 105 trees, each with 125 steps and a consistency index (excluding uninformative characters) of 0.886. The louse analysis yielded 81 trees, each with 64 steps and a consistency index of 0.885. The trees shown in Figure 3 were selected from the set of shortest trees because their topologies are consistent with a locus-by-locus analysis (Baverstock et al., 1979; Hafner et al., 1987) and a UPGMA analysis (Sneath and Sokal, 1973) of the same data sets. In addition, the relationships depicted for both groups are consistent with results of previous systematic investigations of these groups (Hafner, 1982, 1991; Honeycutt and Williams, 1982; Hellenthal and Price, 1984; Price et al., 1985; Hafner et al., 1987).

Although the host and parasite trees illustrated in Figure 3 appear remarkably similar, it is necessary to document that their level of topological similarity exceeds chance expectations. Because the statistical procedures we use to compare tree topologies (Nelson and Platnick, 1981; Hendy et al., 1984) require trees with equal numbers of taxa, we performed two separate tests of topological similarity: in the first, we omitted the louse taxa *Geomydoecus thomomyus* and *G. actuosi;* in the second, we omitted *Thomomydoecus wardi* and *T. minor.* In both cases, the hypothesis of tree independence was rejected ($P < 0.001$ in both cases using the method of Hendy et al. [1984] and $P < 0.01$ in the first case and $P = 0.05$ in the second case using the method of Nelson and Platnick [1981]). Thus, the statistical tests of tree similarity led us to conclude that there has been a general history of cospeciation in this host-parasite assemblage. For the purposes of analysis, we ignored the occurrence of the louse *G. setzeri* in a few populations of the pocket gopher *O. cherriei* (Fig. 3); the methods we use to compare trees can accommodate redundant endemic taxa, such as the lice *G. thomomyus* and *G. actuosi,* but not redundant widespread taxa, such as *G. setzeri* (terminology follows Nelson and Platnick, 1981). Although the presence of *G. setzeri* on *O. cherriei* may result from host-switching (Hafner and Nadler, 1988), Page (1990) provided evidence that the host distribu-

TABLE 1. Matrices of Nei's (1978) unbiased genetic distances for pocket gophers (above) and chewing lice (below) based on analysis of electrophoretic data. Corresponding hosts and their parasites are listed in the same sequence in the two matrices.

Pocket gophers	1	2	3	4	5	6	7	8
1. *Orthogeomys underwoodi*	—							
2. *Orthogeomys hispidus*	0.693	—						
3. *Geomys bursarius*	0.807	0.949	—					
4. *Thomomys talpoides*	1.800	1.825	1.642	—				
5. *Thomomys bottae*	1.800	1.825	1.825	0.949	—			
6. *Orthogeomys cavator*	0.153	0.813	0.964	1.797	1.797	—		
7. *Orthogeomys heterodus*	0.109	0.733	0.869	1.824	1.824	0.141	—	
8. *Orthogeomys cherriei*	0.092	0.662	0.801	1.798	1.798	0.156	0.057	—
Chewing lice	1	2	3	4	5	6	7	8
1. *Geomydoecus setzeri*	—							
2. *Geomydoecus chapini*	0.799	—						
3. *Geomydoecus ewingi*	0.981	1.143	—					
4. *Thomomydoecus wardi*	1.492	1.779	1.086	—				
5. *Thomomydoecus minor*	1.482	1.779	1.086	0.239	—			
6. *Geomydoecus panamensis*	0.170	0.758	0.981	1.204	1.204	—		
7. *Geomydoecus costaricensis*	0.480	0.932	1.143	1.779	1.779	0.480	—	
8. *Geomydoecus cherriei*	0.475	0.823	1.228	1.922	1.922	0.475	0.089	—

tion of *G. setzeri* is more easily explained by descent than by dispersal.

Given statistically corroborated evidence of cospeciation, it is appropriate to ask whether pocket gophers and chewing lice have undergone equivalent amounts of genetic differentiation during their history of parallel phylogenesis. Comparison of the genetic distance matrices for the two groups (Table 1) reveals a highly significant association between corresponding elements in the matrices ($t = 4.113$, $P < 0.01$, when *G. actuosi* and *G. thomomyus* are omitted; $t = 2.815$, $P < 0.05$, when *T. wardi* and *T. minor* are omitted; adjusted $df = 7$ in both cases; Mantel, 1967). Although this is strong evidence that evolutionary rates within each of the groups have been roughly constant through time (the same conclusion reached by Page, 1990), this test reveals nothing about potential rate differences that may exist between the hosts and parasites.

A plot of corresponding genetic distances for hosts and their parasites (Fig. 4) illustrates graphically the relationship between the two matrices (Table 1). Because there is dependence among the elements within each matrix, we are prevented from fitting a line to the points. It is clear, however, that most of them lie roughly equidistant from the two axes, and the array appears to emanate from the vicinity of the origin. The clumping of the points in Figure 4 is an artifact of the branching structure of the host and parasite trees (i.e., the trees lacked branches at the genetic distances corresponding to the gaps). The obvious outlier in Figure 4 is the *Thomomys–Thomomydoecus* comparison, in which the pocket gophers (Nei's $D = 0.949$) are far more differentiated than are their lice ($D = 0.239$). Elsewhere (Hafner and Nadler, 1988), we hypothesized that this represents a case of pseudo-cospeciation, wherein a parasite colonizes a new host that happens to be the sister taxon of the parasite's original host. Although this yields a phylogenetic pattern that is consistent with cospeciation (i.e., sister taxa of parasites are found on sister taxa of hosts), this pattern results from host-switching rather than cospeciation. At present, we have no way of testing whether there is a linear relationship among the points in Figure 4 and, if so, determining confidence intervals for the slope and y-intercept of the line. We point out, however, that the distribution of the points in Figure 4 is consistent with expectations of equal rates of evolutionary

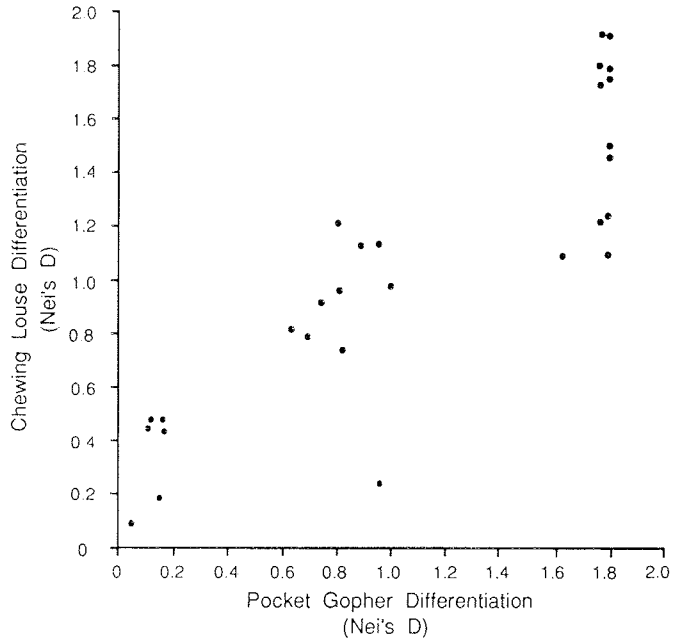

FIG. 4. Bivariate plot of corresponding elements in the pocket gopher and chewing louse matrices of genetic distance (Table 1).

change (Fig. 2a) and synchronous cospeciation (Fig. 2b) in pocket gophers and their ectoparasitic chewing lice.

CONCLUSIONS

We have outlined a method for use of genetic data to investigate cospeciation in host-parasite assemblages. The initial (and most critical) portion of the analysis, documentation of widespread cospeciation, involves a quantitative comparison of host and parasite tree topologies; to this end, use of genetic data to construct the trees has few advantages over other kinds of data used to infer phylogeny. However, given statistical documentation of widespread cospeciation, the availability of genetic data for both hosts and parasites enables a more thorough investigation of the evolutionary history of the assemblage because these data can be converted into estimates of genetic distance to study relative rates of differentiation in the hosts and their parasites.

The method we propose has several limitations, including the problem of large standard errors around estimates of genetic distance based on protein studies. This problem is particularly acute when these estimates are based on examination of a relatively small number of loci, as in our chewing louse example. We are currently obtaining nucleic acid sequence data for pocket gophers and chewing lice, which should increase the resolving power of our test. Also, regardless of the method used to generate the host and parasite trees, there is considerable uncertainty surrounding the topological structure of the trees; this uncertainty will affect the confidence we have in our test for widespread cospeciation. Because the methods we use to compare tree topologies require equal numbers of host and parasite taxa, iterative tests are required in assemblages with redundant endemic parasites; at present, the method cannot accommodate redundant widespread taxa. Finally, the bivariate method we propose to compare genetic distances in hosts and their parasites is not amenable to statistical analysis and, thus, provides

only a heuristic assessment of the relationship between evolutionary rates in the two groups.

In the case study of pocket gophers and chewing lice, we document (within accepted levels of statistical rigor) that these rodents and their ectoparasites share a history of widespread cospeciation. Our comparative analysis of genetic distances in the two groups provides evidence that the rate of genetic change within each group has been roughly constant through time, but results of the between-group analysis of evolutionary rates remain tentative. The bivariate plot of host and parasite genetic distances suggests that pocket gophers and chewing lice may have evolved at approximately equal rates; however, any degree of confidence in this assertion must await development of a rigorous statistical procedure for examining the proportional relationship between genetic distances in the two groups. At present, the data base is too small to restrict the bivariate analysis to phylogenetically independent data points (which would enable use of conventional statistical tests of fit and association). Hence, the addition of new host and parasite taxa to the data base should permit us to examine in greater detail the question of relative rates of genetic evolution in pocket gophers and chewing lice.

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REFERENCES

BAVERSTOCK, P. R., S. R. COLE, B. J. RICHARDSON, AND C. H. S. WATTS. 1979. Electrophoresis and cladistics. Syst. Zool., 28:214–219.

BROOKS, D. R. 1979. Testing the context and extent of host-parasite coevolution. Syst. Zool., 28:299–307.

BROOKS, D. R. 1987. Analysis of host–parasite coevolution. Int. J. Parasitol., 17:291–297.

EICHLER, W. 1948. Some rules in ectoparasitism. Ann. Mag. Nat. Hist., Ser. 12, 1:588–598.

EVELEIGH, E. S., AND H. AMANO. 1977. A numerical taxonomic study of the mallophagan genera *Cummingsiella* (=*Quadraceps*), *Saemundssonia* (Ischnocera: Philopteridae), and *Austromenopon* (Amblycera: Menoponidae) from alcids (Aves: Charadriiformes) of the northwest Atlantic with reference to host-parasite relationships. Can. J. Zool., 55:1788–1801.

FAIN, A. 1979. Specificity, adaptation and parallel host-parasite evolution in acarines, especially Myobiidae, with a tentative explanation for the regressive evolution caused by the immunological reactions of the host. Proc. 5th Int. Congr. Acarol., 2: 321–328.

HAFNER, M. S. 1982. A biochemical investigation of geomyoid systematics (Mammalia: Rodentia). Z. Zool. Syst. Evolutionsforsch., 20:118–130.

HAFNER, M. S. 1991. Evolutionary genetics and zoogeography of Middle American pocket gophers, genus *Orthogeomys*. J. Mammal. (in press).

HAFNER, M. S., J. C. HAFNER, J. L. PATTON, AND M. F. SMITH. 1987. Macrogeographic patterns of genetic differentiation in the pocket gopher *Thomomys umbrinus*. Syst. Zool., 36:18–34.

HAFNER, M. S., AND S. A. NADLER. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature, 332:258–259.

HALL, E. R. 1981. The mammals of North America. Volume 1. John Wiley and Sons, New York. 600 + 90 pp.

HELLENTHAL, R. A., AND R. D. PRICE. 1984. Distributional associations among *Geomydoecus* and *Thomomydoecus* lice (Mallophaga: Trichodectidae) and pocket gopher hosts of the *Thomomys bottae* group (Rodentia: Geomyidae). J. Med. Entomol., 21: 432–446.

HENDY, M. D., C. H. C. LITTLE, AND D. PENNY. 1984. Comparing trees with pendant vertices labelled. SIAM J. Appl. Math., 44:1054–1065.

HONEYCUTT, R. L., AND S. L. WILLIAMS. 1982. Genic differentiation in pocket gophers of the genus *Pappogeomys*, with comments on intergeneric relationships in the subfamily Geomyinae. J. Mammal., 63: 208–217.

HUMPHRIES, C. J., J. M. COX, AND E. S. NIELSEN. 1986. *Nothofagus* and its parasites: A cladistic approach to coevolution. Pages 55–76 *in* Coevolution and systematics (A. R. Stone and D. L. Hawksworth, eds.). The Systematics Association Special Volume No. 32. Clarendon Press, Oxford.

KETHLEY, J. B., AND D. E. JOHNSTON. 1975. Resource tracking patterns in bird and mammal ectoparasites. Misc. Publ. Entomol. Soc. Am., 9:231–236.

KIM, K. C., C. A. REPENNING, AND G. V. MOREJOHN. 1975. Specific antiquity of the sucking lice and evolution of otariid seals. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 169:544–549.

KIMURA, M. 1983. The neutral theory of molecular evolution. Cambridge Univ. Press, Cambridge. 367 pp.

KOJIMA, K., J. GILLESPIE, AND Y. N. TOBARI. 1970. A profile of *Drosophila* species' enzymes assayed by

electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem. Genet., 4:627–637.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27:209–220.

MANTER, H. W. 1955. The zoogeography of trematodes of marine fishes. Exp. Parasitol., 4:62–86.

MOSS, W. W. 1979. Patterns of host-specificity and co-evolution in the Harpyrhynchidae. Proc. 5th Int. Congr. Acarol., 2:379–384.

NADLER, S. A., AND M. S. HAFNER. 1989. Genetic differentiation in sympatric species of chewing lice (Mallophaga: Trichodectidae). Ann. Entomol. Soc. Am., 82:109–113.

NADLER, S. A., M. S. HAFNER, J. C. HAFNER, AND D. J. HAFNER. 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) at a pocket gopher contact zone (Rodentia: Geomyidae). Evolution, 44:942–951.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89:583–590.

NELSON, G., AND N. I. PLATNICK. 1981. Systematics and biogeography: Cladistics and vicariance. Columbia Univ. Press, New York. 567 pp.

PAGE, R. D. M. 1989. COMPONENT user's manual (release 1.5). Univ. Auckland, Auckland, New Zealand. 84 pp.

PAGE, R. D. M. 1990. Temporal congruence and cladistic analysis of biogeography and cospeciation. Syst. Zool., 39:205–226.

PLATNICK, N. I., AND G. NELSON. 1978. A method of analysis for historical biogeography. Syst. Zool., 27: 1–16.

PRICE, R. D., R. A. HELLENTHAL, AND M. S. HAFNER. 1985. The *Geomydoecus* (Mallophaga: Trichodectidae) from the Central American pocket gophers of the subgenus *Macrogeomys* (Rodentia: Geomyidae). Proc. Entomol. Soc. Wash., 87:432–443.

RADOVSKY, F. J. 1979. Specificity and parallel evolution of Mesostigmata parasitic on bats. Proc. 5th Int. Congr. Acarol., 2:347–354.

ROSEN, D. E. 1978. Vicariant patterns and historical explanation in biogeography. Syst. Zool., 27:159–188.

SARICH, V. M. 1977. Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature, 265:24–28.

SCHNELL, G. D., D. J. WATT, AND M. E. DOUGLAS. 1985. Statistical comparison of proximity matrices: Applications in animal behaviour. Anim. Behav., 33: 239–253.

SIMBERLOFF, D. 1987. Calculating probabilities that cladograms match: A method of biogeographical inference. Syst. Zool., 36:175–195.

SIMBERLOFF, D., K. L. HECK, E. D. MCCOY, AND E. F. CONNOR. 1981. There have been no statistical tests of cladistic biogeographical hypotheses. Pages 40–63 *in* Vicariance biogeography: A critique (G. Nelson and D. E. Rosen, eds.). Columbia Univ. Press, New York.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco. 573 pp.

SWOFFORD, D. L. 1989. PAUP: Phylogenetic analysis using parsimony. Version 3.0. Illinois Natural History Survey, Champaign, Illinois. 40 pp.

TIMM, R. M. 1983. Farenholz's Rule and resource tracking: A study of host–parasite coevolution. Pages 225–266 *in* Coevolution (M. H. Nitecki, ed.). Univ. Chicago Press, Chicago.

ZUCKERKANDL, E., AND L. PAULING. 1965. Evolutionary divergence and convergence in proteins. Pages 97–166 *in* Evolving genes and proteins (V. Bryson and H. J. Vogel, eds.). Academic Press, New York.

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APPENDIX 1

Protein loci examined in the electrophoretic survey of pocket gophers were alcohol dehydrogenase (ADH), sorbitol dehydrogenase (SDH), lactate dehydrogenases (LDH-1, LDH-2), malate dehydrogenases (MDH-1, MDH-2), malic enzyme (ME), isocitrate dehydrogenases (ICD-1, ICD-2), glucose dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G6PDH), α-glycerophosphate dehydrogenase (αGPD), superoxide dismutases (SOD-1, SOD-2), glutamate-oxaloacetate transaminases (GOT-1, GOT-2), creatine kinases (CK-1, CK-2), adenylate kinase (AK), 4-methyl-umbelliferyl acetate esterase (EST-D), peptidase A (PEP-A), peptidase B (PEP-B), peptidase C (PEP-C), leucine aminopeptidase (LAP), fumarate hydratase (FUM), aconitases (ACON-1, ACON-2), mannose phosphate isomerase (MPI), glucose phosphate isomerase (GPI), albumin (ALB), and hemoglobin (HB). Loci surveyed in the chewing lice were malate dehydrogenase (MDH), malic enzyme (ME), isocitrate dehydrogenase-1 (ICD-1), xanthine dehydrogenase (XDH), superoxide dismutases (SOD-1, SOD-2), arginine kinase (ARK), 4-methyl-umbelliferyl acetate esterase (EST-D), alpha-naphthyl acetate esterase (EST), peptidase A (PEP-A), peptidase C (PEP-C), adenosine deaminase (ADA), fumarate hydratase (FUM), and glucose phosphate isomerase (GPI).

APPENDIX 2. Allele frequencies at 31 loci in eight taxa of pocket gophers (Geomyidae) and the outgroup, *Dipodomys ordii* (Heteromyidae). Allele frequencies are 1.00 unless otherwise indicated. Locus abbreviations are given in Appendix 1.

	Thomomys		*Geomys*	*Orthogeomys*					*Dipodomys ordii* ($n = 1$)
Locus	*talpoides* ($n = 1$)	*bottae* ($n = 1$)	*bursarius* ($n = 1$)	*hispidus* ($n = 1$)	*cavator* ($n = 4$)	*underwoodi* ($n = 8$)	*cherriei* ($n = 7$)	*heterodus* ($n = 17$)	
ADH	c	d	e	b	a	a	a	a	f
SDH	c	d	a	a	b (0.75) g (0.25)	a	a	a (0.82) e (0.09) f (0.09)	h
LDH-1	c	c	b	b	b (0.50) e (0.50)	b (0.88) d (0.12)	a (0.57) b (0.43)	a (0.88) b (0.12)	f
LDH-2	a	a	a	a	a	a	a	a	b
MDH-1	a	a	a	a	a	a	a	a	a
MDH-2	d	d	c	b	a	a	a	a	e
ME	d	d	c	b	a	a	a	a	c
ICD-1	a	a	a	a	a	a	a (0.93) c (0.07)	a (0.91) b (0.09)	d
ICD-2	e	d	c	b	a	a	a	a	d
GDH	d	c	b	a	a	a	a	a (0.94) e (0.06)	f
G6PDH	e	d	c	a	b	a (0.63) b (0.37)	a (0.43) b (0.57)	b	a
αGPD	e	d	c	b	a	a	a (0.43) f (0.57)	a	g
SOD-1	c	c	b	a	a	a	a	a	d
SOD-2	d	d	c	b	a	a	a	a	d
GOT-1	e	d	a	c	b	a	a	a	f
GOT-2	a	a	a	a	a	a	a	a	a
CK-1	e	d	c	b	a	a	a	a	f
CK-2	e	d	c	b	a	a (0.94) f (0.06)	a	a	e
AK	e	d	c	b	a	a	a	a	f
EST-D	b	b	a	a	a	a (0.75) c (0.25)	a	a	d
PEP-A	h	g	f	e	d (0.75) i (0.25)	c	b	a	j
PEP-B	c	b	a	a	a	a	a	a (0.91) d (0.09)	e
PEP-C	d	d	c	b	a	a	a	a	e
LAP	c	b	a	a	a	a	a	a	d
FUM	b	c	b	a	a (0.88) e (0.12)	a	a (0.72) d (0.28)	a	b
ACON-1	g	f	e	a	d	c	a (0.86) b (0.14)	a (0.91) b (0.09)	a
ACON-2	e	d	c	b	a	a	a	a	f
MPI	c	b	a	a	a	a	a	a	d
GPI	b	c	a	a	a	a (0.88) d (0.12)	a	a	a
ALB	d	c	a	b	a	a	a	a	a
HB	a	a	a	a	a	a	a	a	b

APPENDIX 3. Allele frequencies at 14 loci in 12 taxa of chewing lice (Mallophaga). *Geomydoecus chapini* was mistakenly referred to *G. yucatanensis* in Hafner and Nadler (1988). Allele frequencies are 1.00 unless otherwise indicated. Locus abbreviations are given in Appendix 1.

	Thomomydoecus		*Geomydoecus*								*Neotrichodectes mephitidis*	*Trichodectes octomaculatus*
Locus	*wardi*	*minor*	*thomomyus*	*actuosi*	*ewingi*	*chapini*	*panamensis*	*setzeri*	*cherriei*	*costaricensis*		
MDH	a	a	a	a	a	a	a	a	a	a	a	a
ME	c	c	b	b	b	a	b	b	b	b	c	c
ICD-1	b	b	a	d	d	c	c	c	c	c	e	f
XDH	h	g	f	e	d	b	c	c	b	a	b	b
SOD-1	d	e	b	a	a	c	c	c	c	c	f	g
SOD-2	b	b	a	a	a	a	a	a	a	a	c	d
ARK	b	b	b	b	b	a	a	a	a	a	b	b
EST-D	d	d	c	c	c	c	b	b	a	a	e	f
EST	d (0.50) f (0.50)	d (0.90) f (0.10)	d (0.50) e (0.50)	d (0.48) f (0.47) g (0.05)	d (0.14) f (0.86)	c (0.50) d (0.50)	b (0.50) c (0.50)	a (0.86) b (0.14)	b	b (0.20) d (0.80)	d	d
PEP-A	a	a	a	a	a	b	a	a	a	a	c	d
PEP-C	d	d	a	a	b	e	d	c	a	a	f	g
ADA	b	b	c	c	c	d	a	a	a	a	c	d
FUM	a	a	a	a	a	a	a	a	b	b	c	d
GPI	h	i	e	f (0.10) g (0.90)	f	c	d	a	b	b	h	h